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Award Number: DAMD17-96-C-6128

TITLE: Energy Metabolism in Cold-Stressed Females: Implications for Predictive Modeling (95 Women)

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REPORT DATE: November 1999

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 074-0188*



| 1. AGENCY USE ONLY (Leave blank) | 2. REPORT DATE | 3. REPORT TYPE AND DATES COVERED |
|---|---|---|
| | November 1999 | Final (01 Oct 96 - 01 Oct 99) |

**4. TITLE AND SUBTITLE**
Energy Metabolism in Cold-Stressed Females: Implications for Predictive Modeling (95 Women)

**5. FUNDING NUMBERS**
DAMD17-96-C-6128

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**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

**10. SPONSORING / MONITORING AGENCY REPORT NUMBER**

**11. SUPPLEMENTARY NOTES**

**12a. DISTRIBUTION / AVAILABILITY STATEMENT**
Approved for public release
distribution unlimited

**12b. DISTRIBUTION CODE**

**13. ABSTRACT** ***(Maximum 200 Words)***

Military operations in cold environments have resulted in life-threatening situations if personnel are ill-equipped or unprotected. Prediction of survival time in the cold (*ST*) is essential for search and rescue purposes, to forecast contingencies for operations in such environments, and to evaluate the potential benefits of innovative equipment/clothing designed to protect the soldier from cold. Mathematical models of *ST* are based on the magnitude and capacity for increasing metabolic heat production (*M*) by shivering, and the rate of heat loss from the body. Previous research on males showed that relationships among *ST*, *M*, and heat loss are affected by body composition, the muscle mass recruited, and the type and quantity of substrate oxidized during shivering. Gender-related physiological differences in responses to cold have not been considered in *ST* predictive models. The current research characterized *M* in females during cold stress by quantifying the relative muscle mass recruited during shivering, determining the quantity and quality of energy substrate utilization during shivering, and relating the findings to body temperature regulation. No gender differences were found for *M* and deep body cooling rates if variables such as stature and body adiposity were controlled. Current prediction models of *ST* can be considered gender-invariant.

**14. SUBJECT TERMS**
Womens Health

**15. NUMBER OF PAGES**
224

**16. PRICE CODE**

| 17. SECURITY CLASSIFICATION OF REPORT | 18. SECURITY CLASSIFICATION OF THIS PAGE | 19. SECURITY CLASSIFICATION OF ABSTRACT | 20. LIMITATION OF ABSTRACT |
|---|---|---|---|
| Unclassified | Unclassified | Unclassified | Unlimited |

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# 1 INTRODUCTION

Military units operate in cold air and cold water environments, and the associated training or missions can result in personnel being faced with life-threatening situations if they are ill-equipped or unprotected. As demonstrated by the recent winter crash in the Canadian Arctic of a military aircraft carrying infantry personnel, rescue can be delayed for days even when the precise location of survivors is known (de Groot, 1994). Cold water immersion hypothermia recently caused the deaths during training of US Army Rangers (Fort Benning, 1995). In light of such potential emergencies the prediction of survival time (*ST*) in the cold, defined in this document as the elapsed time until the onset of lethal hypothermia, is essential to meet the needs of Search and Rescue authorities. Such predictions are also useful in the analysis of strategic human factors demands of military operations in the cold, to prepare for contingencies of such operations, and to evaluate the potential benefits of equipment/clothing designed to protect the soldier from the cold.

An understanding of *ST* in healthy, sedentary, non-traumatized individuals is based in the following relationships. Once the protective insulation of available shelter or clothing is maximized, cold-stressed humans elevate metabolic heat production ($\dot{M}$) by shivering in an attempt to balance heat loss. Existing models of *ST* in cold air or cold water are based on observations of factors which affect $\dot{M}$ and the rate of heat loss from the body. In such models $\dot{M}$ increases as a function of temperature signals from the core and skin. When cold exposure is too severe for $\dot{M}$ to balance heat loss, *ST* is largely determined by the rate of heat loss from the body. Where there is a balance between $\dot{M}$ and heat loss, *ST* is limited by the endurance time for shivering.

The physiological factors characterizing $\dot{M}$ are relatively complex. Until about a 15 y ago there was very little empirically based information available in this regard for human subjects. Research has demonstrated that the relationship between *ST*, $\dot{M}$ and heat loss is affected by the extent of the muscle mass involuntarily recruited during

shivering (Bell et al., 1992), connective heat transfer during cold stress (Tikuisis et al., 1991), muscle substrate availability (Jacobs et al., 1994), the type and quantity of substrate oxidized by shivering musculature (Vallerand and Jacobs, 1989), and body composition (Tikuisis et al., 1988). Our research during the last decade has focused on such factors with the objective of generating sufficient knowledge to improve the predictive modeling of *ST* in the cold. A brief review of this research follows.

By measuring the electrical activity of many muscle groups simultaneously during cold-induced shivering, we demonstrated that several large muscle groups are recruited and contract at relatively low intensities that are less than 20% of their maximum force generating capabilities (Bell et al., 1992). Since so many muscle groups are involved in shivering, the sum total of their contractile activities can result in a four or five-fold increase in metabolic rate, and heat production.

Much of our attention has been directed towards the substrates that are used by skeletal muscle to increase heat production during shivering. For example, Vallerand et al. (1988) administered a clinical glucose tolerance test to subjects who were sitting in either cold air or at a comfortable temperature for two hours. These data were the first to show in humans that glucose is eliminated more rapidly from the circulation during cold exposure, presumably to provide more available substrate to fuel the increase in metabolic rate. It is also noteworthy that this more rapid uptake of glucose during cold exposure occurs with lower insulin levels in the cold compared to warm temperatures.

We subsequently continued to attempt to quantify the rates of substrate oxidation of fat, carbohydrate and protein in humans during cold exposure with indirect calorimetric techniques. As one might presume, the increase in metabolic rate during shivering is caused by increases in oxidation of both fat and carbohydrate, but the relative increase in the rate of substrate oxidation caused by shivering is greatest for carbohydrates (Vallerand and Jacobs, 1989). In resting subjects exposed to either cold air or cold water, carbohydrates and fat contribute approximately equally to heat production (Martineau and Jacobs, 1991; Vallerand and Jacobs, 1989). From a strategic point of view, this finding seems unfortunate because the body's availability of carbohydrates is quite limited compared to the abundant fat and protein stores. We were already aware of the well established positive relationship between muscle glycogen

concentration and endurance exercise performance of skeletal muscle and speculated that there may be a similar detrimental effect caused by muscle glycogen depletion on another form of muscle contraction, i.e. shivering and the associated heat production.

We therefore carried out a series of studies on male subjects immersed in 18°C water. The subjects were removed from the water when their rectal temperature reached 35.5° C. Biopsies were taken from the thigh muscle before and after the immersion to evaluate the changes in glycogen as a result of the water immersion (Martineau and Jacobs, 1988). In another study muscle glycogen concentrations were manipulated prior to water immersion by appropriate dietary and exercise protocols (Martineau and Jacobs, 1989); the purpose of these studies was to evaluate the effects of very low and very high glycogen levels on metabolic heat production during the water immersion.

Metabolic rate during cold water immersion, expressed as oxygen consumption, increases to values that are usually around 4 or 5 times normal resting metabolic rate. Infrequently we have observed individuals who exhibit somewhat higher values, 6– or 7 times resting values. Our initial studies suggested that part of this increase in metabolic rate is fueled by muscle glycogen, as all of the subjects demonstrated a decrease in leg glycogen concentration after the water immersion (Martineau and Jacobs, 1988). The second objective of these experiments was to evaluate the effects of manipulating the pre-immersion glycogen levels on heat production during cold water immersion. Our manipulations did result in the subjects entering the water on one trial with muscle glycogen levels that were only about 50% of normal, and on another trial when they were about 150% of normal (Martineau and Jacobs, 1989). The oxygen consumption during the water immersion, was about the same on each trial. The respiratory exchange ratio (RER), however, differed between trials as expected. Metabolic heat production is calculated based on the combination of RER and oxygen consumption. We observed significantly less metabolic heat production per unit time when the body's carbohydrate stores were depleted compared to the other trials (Martineau and Jacobs, 1989). There was also a significantly more rapid body cooling rate, as reflected by the changes in rectal temperature, when the body had little glycogen stored in its muscles, and presumably also in the liver.

These examples of some of our initial studies were done on subjects resting in cold air or cold water. In light of these findings we hypothesized that the requirement to do physical work superimposed on that cold stress might induce a more rapid breakdown of muscle glycogen than if the same work were done at a comfortable temperature. We therefore had subjects performing either light or heavy exercise once at 9°C air and again on a separate day at 21°C (Jacobs et al., 1985). We found that significantly more glycogen was in fact utilized to do the light exercise in the cold compared to doing the same work at 21°C. There was no difference in glycogen depletion rates, however, for the higher exercise intensities, and this is consistent with earlier observations that the heat production associated with hard exercise is sufficient to offset heat loss to the environment, thus obviating the need for shivering (Hong and Nadel, 1979).

We also carried out investigations of the effects of manipulating the body's circulating fat pools on heat production during cold water immersion. Vallerand and Jacobs (1990) reported that triglycerides infused intravenously were not eliminated more rapidly from the circulation during cold air exposure than during warm air exposure, contrasting with the results for glucose infusion (Vallerand et al., 1988). In another series of experiments, the circulating free fatty acid concentration was manipulated by having our subjects ingest nicotinic acid in the form of niacin pills prior to and during the water immersion (Martineau and Jacobs, 1989b). The effect of the nicotinic acid is to block lipolysis and this effect is demonstrated by the observation that the plasma free fatty acids and glycerol levels were dramatically reduced prior to, and during, the water immersion. Again contrasting with the effects of manipulating the carbohydrate stores, metabolic heat production was virtually unaffected; the proportion of the total heat production that could be attributed to fat oxidation was significantly reduced, but there was compensation by simply increasing carbohydrate oxidation.

For reasons that are still unclear, carbohydrates seem to be a somewhat preferred substrate during shivering thermogenesis. There are similarities to hard physical exertion in that the body is not able to maintain the same intensity of exertion when carbohydrate stores are depleted, i.e. a shift to a greater reliance on fat oxidation to fuel muscle contraction is not sufficient for the musculature to be able to maintain a high level of exertion, just as body temperature could not be maintained as well when carbohydrate

stores were depleted (Martineau and Jacobs, 1989a). We must mention that similar experiments were carried out at USARIEM and they did not detect any significant muscle glycogen utilization during cold water immersion (Young et al., 1989); we can not explain the discrepancies between our studies other than to suggest that perhaps the fact that our subjects were much leaner than those of Young et al. (1989) may be important in this regard.

Gender differences in response to cold stress have been the topic of a limited number of investigations and reviews (Stephenson and Kolka, 1993; Nunneley, 1978; Hayward et al., 1975). It was reported that women cool faster than men during cold water immersion (Kollias et al., 1974; McArdle et al., 1984; Hessemer and Brück, 1985), and this is somewhat surprising considering the greater body fat content of the average female. Body temperature changes associated with the menstrual cycle (Graham et al., 1989), cardiovascular responses to rest and exercise (Stevens et al., 1987; Wagner and Horvath, 1985a,b) are other factors with associated gender differences in response to cold stress. To date potential gender-related physiological differences in responses to cold have not been considered in systematic studies such as those described above, i.e. quantification of the substrates used to fuel $\dot{M}$ during cold stress, nor in the development of $ST$ predictive models, including our own [Tikuisis, 1989; Tikuisis et al., 1988). Specifically, there are established gender differences in the ratio of lean body mass to total body mass and in the proportion of energy derived from carbohydrate or fat metabolism during exercise (Tarnopolsky et al., 1990). There are, however, studies of gender differences with regard to skeletal muscle metabolism during exercise which suggest that untrained female musculature has an enzymatic profile which is predisposed to greater dependency on lipid metabolism than male muscle tissue (Green et al., 1984). In male and female subjects matched for their physical training status, exertion at the same relative intensity is fueled by carbohydrate oxidation to a greater extent in males, and by lipid oxidation to a greater extent in females (Tarnopolsky et al., 1990; Phillips et al., 1993; Tarnopolsky et al., 1995). Although potentially advantageous for endurance exercise, the evidence presented above relating to the importance of carbohydrate oxidation for shivering thermogenesis suggests that less carbohydrate oxidation may be disadvantageous in terms of $ST$ in the cold. However, even if the magnitude of the

increase in $\dot{M}$ may be less in females than males, the metabolic predisposition favoring lipid oxidation suggests that temperature regulation may not be as negatively influenced when glycogen availability is compromised. In terms of the muscle mass involved in shivering, models of human thermoregulation during cold stress use a fixed value to represent the contribution of the musculature of various body segments to the increase in $\dot{M}$ due to shivering. For example, this constant for the contribution of the trunk has previously only been estimated and ranged from 55-85% (Montgomery, 1974; Stolwijk, 1970; Hancock, 1980). We recently experimentally determined this value for male subjects to be 71% (Bell et al., 1992), but here again no data are yet available for female subjects. The implications of these gender differences, if they apply to cold-induced increases in $\dot{M}$, are potentially of sufficient magnitude to warrant their consideration in a model of *ST* in cold stressed females.

## 1.1 Technical Objectives

The proposed research is delimited in scope to addressing metabolic heat production and substrate utilization during cold stress. The effects of the menstrual cycle in this regard are not addressed in this research. Experiments were designed to quantify the relative muscle mass recruited during shivering, and to determine the quantity and quality of energy substrate utilization in female subjects when metabolic rate is increased during cold stress. This research was conducted with a view to relating this information to body temperature regulation during cold stress in cold air and cold water. The data generated from these experiments were analyzed with existing mathematical models of *ST* in the cold in order to determine if a gender-specific factor should be incorporated into the metabolic heat production component, i.e. $\dot{M}$.

Three studies were proposed; all were carried out only on female subjects and the results were compared with data from male subjects that were already collected and published.

**Study 1, "Quantification of carbohydrate and lipid oxidation rates in shivering females ,"** was designed to clarify the quantity and quality of energy substrate utilization in shivering female subjects during cold water immersion. The objectives

were: to determine the magnitude of $\dot{M}$ during cold water immersion; to quantify the relative contributions of fat, carbohydrate and protein metabolism to fueling $\dot{M}$ ; to determine if muscle glycogen is a significant energy source during shivering; to manipulate skeletal muscle glycogen availability and to determine the subsequent effects on $\dot{M}$ and body temperature regulation during cold stress. The comparative data for male subjects were taken from Marineau and Jacobs (1989).

**Study 2, "Effects of exercise when cold-stressed on substrate utilization,"** was designed to compare substrate utilization during light and moderate exercise performed in both cold and comfortable ambient temperatures. The objectives were: to quantify $\dot{M}$ , lipid and carbohydrate oxidation rates, and muscle glycogen utilization during light and moderate exercise in the cold; to compare the results to when the same exercise is performed at comfortable temperatures. The comparative data for male subjects were taken from Jacobs et. al. (1985)

**Study 3, "Relative intensity of muscular contraction during shivering,"** was designed to document the intensity of muscular contraction during shivering in a variety of skeletal muscle groups chosen to represent both central and distal body regions. This information was needed to clarify the extent of the contribution of various body parts to total heat production during shivering. Electromyography (EMG) was used to monitor muscle electrical activity during a two hour cold air exposure and the EMG was compared to the EMG that was generated during maximal voluntary contractions of the same muscle groups so that the relative intensity of muscle contraction during shivering could be quantified. Simultaneous measurements of EMG, respiratory gas exchange, body temperatures, and heat flux were used to clarify the association among shivering, $\dot{M}$ , and body temperature regulation. The comparative data for male subjects were taken from Bell et al. 1992.

## 1.2 Hypotheses/Purposes

**Study 1:** The relative increase in metabolic heat production ( $\dot{M}$ ) caused by cold water immersion will be less in females than what has previously been reported for males. Compared to previous reports for males, a smaller proportion of the increase in

$\dot{M}$ will be due to increases in carbohydrate oxidation. Unlike what has been reported for males, muscle glycogen depletion of large skeletal muscle groups will not impair body temperature regulation during cold water immersion.

**Study 2:** $\dot{M}$ will increase to a greater extent when light exercise is performed during cold air exposure than when performing the same exercise at a comfortable temperature. Contrasting with previous reports for male subjects, this increase will not be associated with any greater muscle glycogen utilization than is the case when the same exercise is performed at a comfortable temperature.

**Study 3:** This study is descriptive thus no experimental hypotheses were postulated *a priori*. One purpose of the study was to document the relative intensity of muscular contraction during shivering in a variety of skeletal muscle groups chosen to represent both central and distal body regions. Another purpose was to determine the association in females between the observed increase in muscle electrical activity and the increase in $\dot{M}$.

## 2 ABSTRACTS

### Study 1: Quantification Of Carbohydrate And Lipid Oxidation Rates In Shivering

This document is a progress report which describes the results from the first of a series of studies carried out to clarify the extent of gender-related differences in physiological responses to cold stress, and to evaluate the potential implications for survival time in the cold. Specifically, this study was designed to clarify the quantity and quality of energy substrate utilization in shivering female subjects during cold water immersion. The objectives were: to determine the magnitude of metabolic heat production during cold water immersion; to quantify the relative contributions of fat, carbohydrate and protein metabolism to fueling metabolism; to determine if muscle glycogen is a significant energy source during shivering; to manipulate skeletal muscle glycogen availability and to determine the subsequent effects on metabolic rate and body temperature regulation during cold stress. Female subjects were immersed to the neck in 18°C water for up to 90 minutes. Their metabolic rate increased to about 3 times resting levels, similar to what was reported previously for male subjects. About 40% of the

metabolic heat production during immersion was fueled by oxidation of carbohydrates, somewhat less than what was reported for male subjects. Also, as reported earlier for males, muscle glycogen decreased significantly during the immersion suggesting that it is a significant constituent of the carbohydrate component of metabolic heat production during shivering. The implications of manipulating glycogen availability in females could not be adequately addressed because the protocol did not result in the desired changes in muscle glycogen prior to immersion.

**Study 2: Effects Of Exercise When Cold-Stressed On Substrate Utilization**

This document is a progress report which describes the results from the second of a series of studies carried out to clarify the extent of gender-related differences in physiological responses to cold stress, and to evaluate the potential implications for survival time in the cold. Specifically, this study was designed to compare substrate utilization during light (LI) and moderate intensity (MI) exercise performed in both cold and comfortable ambient temperatures. The objectives were to quantify metabolic rate ($\dot{M}$), lipid and carbohydrate oxidation rates, and muscle glycogen utilization during light and moderate exercise in the cold versus comfortable ambient temperatures, and to compare these results with those previously collected in males who underwent the same protocol. Sixteen female subjects were divided into two groups matched for the submaximal exercise intensity corresponding to a blood lactate concentration of 4 mM (W4) during an incremental cycle exercise test. On two separate days subjects rested for 30 min at ambient temperatures of either 9 or 21°C, with the order of the trials balanced among subjects. Following rest a muscle biopsy was obtained from the m. vastus lateralis. Subjects in the MI group exercised for 30 min at 60%W4 while subjects in the LI group exercised for 30 min at 30%W4. Subjects exercised at the same power output for both trials. Another biopsy was taken immediately after exercise and both samples were assayed for glycogen concentration. $\dot{M}$ was significantly higher (13%) during exercise in the cold in LI but not MI. Muscle glycogen decreased significantly in MI (-28%) but not in LI; ambient temperature did not affect the magnitude of the change in

muscle glycogen in either group. The relative contributions of carbohydrate, fat and protein oxidation to fueling $\dot{M}$ were similar in each exposure for both groups. These data provide further evidence of a more pronounced energy metabolism "predisposition" towards lipid metabolism in females than in males.

### Study 3. Relative Intensity Of Muscular Contraction During Shivering

One purpose of the study was to document the relative intensity of muscular contraction during shivering in a variety of skeletal muscle groups chosen to represent both central (pectoralis major (PE), rectus abdominis (AB)), and distal (biceps brachii (BB), brachioradialis (BR), rectus femoris (FE), and gastrocnemius (GA)) body regions in order to clarify the extent of the contribution of various body parts to total heat production during shivering. Another purpose was to determine the association in females between the observed increase in muscle electrical activity and the increase in $\dot{M}$. Fifteen females, (28±6 y; 162.8±4.8 cm; 63.9±8.7 Kg, 23.2±4.7% fat) of normal fitness were recruited. Electromyography was used to monitor muscle electrical activity during a two-hour cold air exposure and the EMG was compared to the EMG that was generated during maximal voluntary contractions of the same muscle groups so that the relative intensity of muscle contraction during shivering could be quantified. Simultaneous measurements of EMG, respiratory gas exchange, body temperatures, and heat flux were used to clarify the association among shivering, $\dot{M}$, and body temperature regulation. Shivering intensity was greatest in the central versus the peripheral muscles. In fact, AB and PE accounted for 84.5% of whole body measured EMG. There was no significant effect of cold exposure on EMG measured at BR or GA. Shivering intensity did not change during the course of the cold exposure. Similarly, in males, EMG activity was also greatest in the trunk (PE and AB contributing 70.5%). In contrast to females, in males, shivering EMG was significantly higher than pre-exposure in all muscle groups measured and continued to increase throughout the exposure. Both males and females showed a strong positive correlation between metabolic heat production per unit lean body mass and whole body shivering.

# 3 METHODS

A brief description of the methodology used in of each study follows. For more detail please see the respective Annex.

## 3.1 Study 1: Quantification Of Carbohydrate And Lipid Oxidation Rates In Shivering Females

The protocol and methodology were chosen to enable comparison with data collected for male subjects using similar protocols (Martineau and Jacobs, 1989). Nineteen female subjects, aged 19-37, were recruited from local universities and within our research facility. Subjects did not donate blood for 30 days prior to or during participation in this study.

Subjects reported for their first visit having read a detailed information summary about all aspects of the study. They were given an opportunity to ask questions of the Scientific Authority and medical officers. Subjects then signed an informed consent and underwent a medical screening. Once receiving medical clearance, physical characteristics including height and weight were determined and percent body fat was estimated after determination of body density by hydrostatic weighing. Maximum aerobic power ( $\dot{V}O_{2max}$ ) was assessed separately for both arm cranking and leg cycle ergometry so that workrates for glycogen depletion exercise of both the upper and lower body could subsequently be determined. To familiarize the subjects with the laboratory setting and test procedures they were also immersed to the shoulders in 18°C water for 15 min.

### 3.1.1 Experimental design.

On three subsequent visits the subjects were immersed in 18°C water, with identical procedures and measurements occurring for each cold exposure. The first control immersion was done after a 3-day period during which the subjects consumed an uncontrolled mixed diet (C). The two other experimental immersions followed 2.5 days of a specific dietary and/or exercise regimen (as described below) designed to elicit low

(L) or high (H) glycogen levels in large skeletal muscle groups. The order of these dietary manipulations was counterbalanced among the subjects, with at least 6 days of uncontrolled mixed diet given between the two dietary regimes.

### 3.1.2 Glycogen depleting exercise and dietary manipulations.

Three days before the two experimental immersions (L or H), two-legged exercise was performed for 60 min on a cycle ergometer at a power output requiring about 70% $\dot{V}O_{2max}$. This was followed by three or four 5-min exercise bouts performed at about 90% $\dot{V}O_{2max}$. This procedure has been used previously to lower the glycogen content of all muscle fiber types of the leg muscles. After a 30-min rest period, the same exercise protocol was repeated using an arm-crank ergometer; the corresponding exercise intensities were about 60 and 90% arm $\dot{V}O_{2max}$. Immediately at the end of the exercise sessions, the subjects were instructed and informed about the composition of different types of food associated with carbohydrate (CHO)-reduced and CHO-rich diets. A list of suggested foods and a sample meal plan was provided to each subject. For 2.5 days the subjects consumed a free choice of foods within these guidelines. They were instructed verbally and with written guidelines that CHO should make up 90-95% of total nutritional consumption during the CHO-rich diet, but only 5-10% in the CHO-poor diet. The subjects were asked to try to maintain the same total caloric take as usual throughout the diet period to ensure that body weight was being maintained. Previously, this laboratory used the same protocol to significantly alter the glycogen content in the vastus lateralis in males subjects. Body weight was determined prior to each immersion.

### 3.1.3 Cold water immersion.

On the day of each immersion the subjects reported to the lab in a 12-h post absorptive state, clad in a two-piece bathing suit. They inserted a rectal probe, were instrumented with 12 calibrated heat-flow transducers, bipolar ECG skin electrodes, and an intravenous catheter. They lay quietly in a supine position for 30 min at 23°C. Their resting metabolic rate was determined during the final 10 min of rest. They then entered the immersion tank and remained in a supine position immersed to the neck in stirred chilled water for up to 90 min or until rectal temperature decreased to 35°C. Muscle tissue from the vastus lateralis and venous blood samples were obtained after the 30 min rest period

and immediately after each cold water immersion. Blood samples were also obtained after 5, 30, 60, and 90 min of immersion. During the immersions the following were averaged each minute: rectal temperature, mean skin temperature and mean skin heat flow, oxygen uptake, and carbon dioxide production.

### 3.1.4 Biological samples.

The subjects were asked to collect their urine for 24 hours beginning the morning of each immersion day. Urine was assayed for urea nitrogen excretion. The blood samples, drawn from a venous catheter in a superficial forearm vein, were assayed for glucose, lactate, FFA, glycerol, epinephrine, norepinephrine, insulin, glucagon, hemoglobin and hematocrit. Muscle samples, obtained under local anaesthetic using a needle biopsy technique, were analyzed for glycogen and triglyceride concentrations. The metabolic heat production, fat and carbohydrate oxidation rates were estimated from the oxygen consumption and non-protein respiratory exchange ratio measurements (RER).

### 3.1.5 Statistics.

The results of the present study were divided into two parts for the purpose of statistical analysis and presentation of results. The first part consisted of the control immersion data alone and was analyzed using a one-factor analysis of variance for repeated measures to determine the main effects of immersion (pre vs. post and/or during). Of the twelve subjects completing the control immersions, only 10 completed 85 minutes of immersion and two asked to terminate immersion after 60 min. Thus, data are presented in two groups; a group that completed 85 minutes of the control immersion (C85), n=10, and a group that completed at least 60 min of immersion (C60), n=12.

The second part involved analysis of the effect of treatment and includes data from the control immersion and the low and high carbohydrate immersions. These results were analyzed with a two-factor analysis of variance for repeated measures to determine the main effects of the immersion (pre vs. post and/or during) and diet (L, C, or H). Of the nine subjects that participated in both treatment immersions, one had completed only 60 min of the control immersion. Therefore, these subjects were divided into two groups for the purpose of data analysis and presentation. One group completed

at least 60 min of each trial (T60), n=9, and the other group completed 85 min of every trial (T85), n=8. Statistical analysis was only performed on the groups that completed 85 min (C85 and T85). Statistical significance was accepted at the 95% confidence level.

## 3.2 Study 2: Effects of Exercise When Cold-Stressed on Substrate Utilization

The protocol and methodology were chosen to enable comparison with data collected for male subjects using a similar protocol (Jacobs et al. 1985). To facilitate these comparisons we restricted our metabolic studies to the use of indirect calorimetry, measurement of hormones and metabolites in venous blood, and measurement of metabolites in muscle biopsy samples.

Sixteen female subjects, aged 19-37, were recruited from local universities and within our research facility. Subjects reported for their first visit having read a detailed information summary about all aspects of the study. They were given an opportunity to ask questions of the Scientific Authority and medical officers. Subjects then signed an informed consent and underwent a medical screening. Once receiving medical clearance, physical characteristics including height and weight were determined and percent body fat was estimated after determination of body density by hydrostatic weighing. Subjects performed an exercise test to near exhaustion on an electrically braked cycle ergometer to determine the power output at a lactate concentration of 4 mmol/l (W4). W4 is an index of aerobic fitness and exercise intensity (Jacobs et al. 1995; Jacobs, 1986) and was used in this study to determine the exercise intensity at which subjects in each group would perform. During this exercise test, subjects began cycling at 30 or 60 Watts and intensity was increased by 30 watts every 4 min. Blood was drawn from the ear lobe during the last 30 s of each 4 min interval. W4 was interpolated from a plot of lactate concentration against power output. Metabolic measurements were made throughout the test.

### 3.2.1 Experimental design

The subjects were divided into two groups, equally matched for the W4 scores. The low intensity (LI) group exercised at 30% W4 and the moderate intensity (MI) group exercised at 60% W4. On two subsequent visits the subjects exercised for 30 min

following a 30 min supine rest, once at a room temperature of 9°C and once at 21°C, with identical procedures and measurements occurring for each exposure. The order of the exposures was counter-balanced among subjects with at least 7 days between trials.

### 3.2.2 Exercise and Exposure

On the day of each exposure the subjects reported to the lab in a 12-h post absorptive state, clad in shorts, t-shirt socks and running shoes. They inserted a rectal probe, were instrumented with 12 calibrated heat-flow transducers, bipolar ECG skin electrodes, and an intravenous catheter. They lay quietly in a supine position for 30 min at either 9°C or 21°C. Their resting metabolic rate was recorded for 10 minutes beginning after 15 minutes of rest in a supine position using a semi-automated metabolic cart system. Rectal temperature, heat flow and skin temperatures were also measured during this time. Venous blood samples were obtained from an antecubital vein just before and after the 30 min rest and at 15 and 25 min during exercise. The first biopsy was taken after these measurements were made, after which the subjects mounted the cycle ergometer and cycled for 30 min at an intensity of either 30% or 60% of W4 depending on the group to which they were assigned. Metabolic rate, heat flow and skin temperature were measured continuously during the 30 min of exercise. Heart rate and ratings of perceived exertion were recorded every 5 min.

### 3.2.3 Statistical analyses

Comparisons between the LI and MI groups were made using a one-factor analysis of variance while intra-group comparisons were made using a repeated measures analysis of variance. It was decided *a priori* that statistical significance would be accepted at the 95% confidence level.

## 3.3 Study 3: Relative Intensity Of Muscular Contraction During Shivering

The protocol and methodology were chosen to enable comparison with data collected for male subjects using a similar protocol (Bell et al. 1992). Sixteen subjects reported for their first visit having read a detailed information summary about all aspects of the study. They were given an opportunity to ask questions of the medical officers.

Subjects then signed an informed consent and underwent a medical screening. Once receiving medical clearance, physical characteristics including height and weight were determined and percent body fat was estimated after determination of body density by hydrostatic weighing. Maximal oxygen uptake was determined during an exercise test to exhaustion on an electrically braked cycle ergometer. Subjects began cycling at 60 Watts and intensity increased in a ramp fashion by 20 watts every min. Metabolic measurements were made continuously throughout the test as described below. Subjects then performed maximal voluntary isometric contractions with each muscle group as they would on the day of the experiment (see below) in order to familiarize the subject with the procedures and muscle contractions required for MVCs.

### 3.3.1 Exposure

On the day of each exposure the subjects reported to the lab in a 12-h post absorptive state, clad in a two piece swimsuit or similar attire. Each experiment consisted of a single exposure to 10°C air at about 40% relative humidity for 2 h. Subjects were asked to inform us on the first day of their menstrual cycle so that their experimental session could be scheduled to occur during the follicular phase. Before experiments, each subject was fitted with EMG electrodes on one side of their body to monitor the EMG of the muscles described below. The other half of their body was instrumented with 12 heat flow transducers for both heat flow and skin temperature measurements. The subjects also inserted a rectal thermistor, and an intravenous catheter was inserted into an antecubital vein.

### 3.3.2 EMG.

The EMG electrodes were placed 0.03 cm apart on the mid-belly of six muscle sites representing the trunk and limbs: pectoralis major (PE), rectus abdominis (AB), biceps brachii (BB), brachioradialis (BR), rectus femoris (FE), and gastrocnemius (GA). The signals were amplified 2000 times (1000 times at the pre-amplifier and twice at the unit) filtered with a band width of 8-500 Hz, integrated, and averaged every 100 ms (ME-3000P-8, Biomation, Almonte, Ontario, Canada). The subject performed a series of voluntary isometric contractions with each muscle so that the regression of EMG against

force could be plotted and calculated for each subject. Force was measured using a 0-1000lb load cell (LPT-1000, BLH Electronics Canada, Toronto, Ontario, Canada) and displayed (LCP-100, BLH Electronics Canada, Toronto, Ontario, Canada) such that the subject could see the digital output in kilograms. The series consisted of three consecutive 5-8 s contractions at different levels of force. The time between each contraction was approximately 1 min. Subjects performed a maximal voluntary contraction (MVC) three times. Of the three MVC values, the one corresponding to maximal force also elicited the highest EMG. Subjects were then asked to produce contractions at 50, 20, and 10% and 5% of the highest MVC.

After these measurements the subjects, wearing only a two-piece bathing suit or similar attire, assumed a standard supine position with arms and legs spread apart on a rope mesh cot and were wrapped in blankets to maintain thermal comfort in a thermal neutral room (21°C). During 15 to 25 min of this 30 min period, the subject's resting metabolic rate, core temperature, skin temperature and heat flow were determined. At the end of this period, the subject was wheeled into the cold chamber, blankets were removed, and the 2-h cold exposure began. Core temperature, heat flow, skin temperatures and EMG activity were recorded continuously and respiratory gas exchange variables were recorded during the periods 5-30, 40-60, 70-90, and 100-120 min. The subject was asked to refrain from voluntary movements except during 1 min stretch breaks allowed every 15 min. Venous blood samples were obtained from an antecubital vein just before and after the 30 min rest and immediately post exposure.

### 3.3.3 Treatment of data

Integrated EMG data were averaged over each minute. Four minute intervals around the stretch break were not included in the analysis. Resting EMG was subtracted from all values to provide only the non-resting portion of the EMG. Therefore, in this report, EMG refers only to the non-resting component of EMG activity. In order to determine shivering intensity, EMG during shivering was expressed as a percentage of the EMG during the highest recorded MVC. Since the relationship between EMG and force is often non-linear (Bigland-Ritchie, 1981; Bell et al. 1992), separate calibration factors were applied. These factors for each subject were obtained from a linear fit of the EMG-force relationship of each muscle between 0 and 20% MVC with the assumption that the

type of contraction during shivering is the same as that during an isometric MVC. Thus, EMG multiplied by this calibration factor indicates the EMG for the corrected amount of force that is produced relative to the MVC of the muscle and is referred to as EMG(%MVC). A whole body index of shivering intensity (SUM) was determined by multiplying EMG(%MVC) by the relative mass of each respective muscle.

$$SUM = \sum_{i=1}^{6} m_i \bullet EMG(\%MVC)_i \qquad (1)$$

where $m_i$ is the muscle mass fraction of the body represented by muscle *i*. Composition analysis of cadavers (Clarys and Marfell-Jones, 1986) was used to determine limb muscle mass factors and the trunk muscle mass factors were obtained from two anthropometric studies (McConville et al. 1980, Snyder et al. 1975). Muscle mass factors were 0.34 for PE (upper trunk), 0.19 for AB (lower trunk), 0.06 for BI (upper arm), 0.035 for BR (lower arms), 0.29 for FE (upper legs) and 0.085 for GA (lower legs). Finally, a specific muscle's contribution to overall body shivering (%Contribution) was determined by dividing the relative shivering intensity of that muscle by SUM.

$$\%Contribution_i = \frac{m_i \bullet EMG(\%MVC)_i}{SUM} \qquad (2)$$

Previously, shivering onset times for various muscles in males were subjectively determined by noting when the EMG signal spiked relative to the background signal. Current EMG measurement technology provides signals that are omnipresent and with greater continuity, thus making it more difficult and arbitrary to select an actual shivering onset time. A more objective determination of shivering onset was sought in the present study that would allow gender comparisons to be made without bias. The objective measure chosen was the half time ($t_{1/2}$) of shivering response. Although this does not provide an onset time *per se*, it does provide a measure of the time to steady state shivering (note that 95% of steady state is attained within 5 half times).

$$EMG_{shiv} = EMG_{ss} \cdot [1 - \exp(-0.693 \cdot t/t_{1/2})] \qquad (3)$$

where $EMG_{ss}$ represents the steady state shivering level (assumed equal to the mean $EMG_{shiv}$ over the last 15 min of the 2 h exposure). The above equation was regressed against the male data for each muscle separately. This regression was then repeated for the female data using the male-fitted values of $t_{1/2}$ as the initial estimate of the half time. The F-ratio test at the acceptance level of 0.05 was used to determine whether the regressed fit of t1/2 was significantly different between genders.

### 3.3.4 Statistical analyses

The effect of exposure on in the cold, data were analyzed using a repeated measures analysis of variance. Unless otherwise noted, data are presented as mean values ±SD. It was decided *a priori* that statistical significance would be accepted at the 95% confidence level.

## 3.4 Instrumentation, measurements and analytical techniques for all studies:

The protocols and methodologies were chosen to enable comparison with data collected for male subjects using similar protocols (Martineau and Jacobs, 1989; Jacobs et al. 1985, Bell et al. 1992). To facilitate these comparisons we restricted our metabolic studies to the use of indirect calorimetry, measurement of hormones and metabolites in venous blood, and measurement of metabolites in muscle biopsy samples.

Subjects did not donate blood for 30 days prior to or during participation in this study. They were asked to abstain from alcohol for 48 hours before a trial, not exercise within 24 hours of a trial, and fast for 12-14 hours before each experimental trial. The subjects were tested at the same time of day to avoid possible diurnal effects.

Initially, there was an attempt to have subjects commence experimentation during the follicular phase of their menstrual cycle. However, for Studies 1 and 2 that involved several experimental trials, some subjects would wait two to three months to complete testing if we controlled for menstrual cycle phase. This would have threatened subject compliance. Since there is no known effect of menstrual cycle on thermoregulation or substrate utilization, the decision was made not to control for menstrual cycle. In Study 3 all subjects were tested in the follicular phase of their menstrual cycle.

### 3.4.1 Muscle biopsies

Muscle samples were taken from the right *quadriceps femoris vastus lateralis* employing the percutaneous needle biopsy technique (Bergström, 1962). Skin and the underlying fascia were anaesthetized with 3 mL of xylocaine (2% epinephrine) after cleansing with an antiseptic solution (Betadine surgical scrub, Purdue Frederick Inc.). Both pre- and post exposure samples were taken from the same incision. Incisions were closed using Steri-Strip® (3M, St. Paul, MN). An elasticized bandage was wrapped around the thigh in an attempt to exert some pressure on the biopsy site and hopefully reduce the soreness that is frequently experienced in the thigh for 2-3 days after the biopsy. This bandage was left on the leg during the exposure, removed for the post-exposure biopsy and then dry Steri-Strips® and a dry elasticized bandage were placed on the leg after the experiment. Subjects were instructed to leave the elasticized bandage on the leg for 3-4 hours; they were instructed to leave the Steri-Strips® in place for 5 days. During the subsequent exposure, incisions were made on the same leg but at least 3 cm away from the previous incision.

### 3.4.2 Blood sampling

Difficulties in obtaining sufficient volume of blood were sometimes encountered during the cold exposure, probably due to the combination of vasoconstriction and decreased blood flow to the forearm. After trying several methods (see Methods, 4.7 Blood sampling, Annex 1), the best results were achieved using a heparin lock (10 U/mL) with the 20 gauge 1 inch catheter. This method was used for all three studies. A waterproof dressing (Tegaderm®) was placed over the site where the catheter pierced the skin to help stabilize the catheter. Required blood samples were drawn into tubes which were kept on crushed ice: 5 mL were expelled into a tube treated with EGTA (90 mg/mL) and glutathione (60 mg/mL), centrifuged and the plasma was frozen for subsequent determination of catecholamines; 5 mL were dispensed into a chilled, EDTA-treated tube (50 µL were dispensed into tubes containing $HClO_4$ for the subsequent determination of glucose and lactate; samples were taken to determine hematocrit and hemoglobin; the

remainder was centrifuged and aliquots of the plasma was subsequently used for the determination of free fatty acids and glycerol). All samples were stored at -20°C until frozen and then stored at -70°C until assayed.

### 3.4.3 Biochemistry

Hematocrit was determined by centrifugation (Autocrit Ultra3 centrifuge). Commercially available kits were used to measure concentrations of free fatty acids (WAKO NEFA kit, Texas). Glucose and hemoglobin were assayed using automated spectrophotometric techniques (Hemocue AB; Helsingborg, Sweden). After deproteinization samples were analyzed for glycerol (Boobis and Maughan, 1983) and lactate (Maughan, 1982). Plasma epinephrine and norepinephrine levels were measured using negative ion chemical ionization gas chromatography-mass spectrometry (Zamecnik, 1997). Changes in plasma volume were calculated from the changes in hematocrit and hemoglobin concentration (Dill and Costill, 1974).

Muscle tissue samples were freeze dried under vacuum for at least 8 hours. Glycogen was assayed as glucose units following hydrochloric acid hydrolysis using a fluorometric enzymatic method (Karlsson, 1971).

To facilitate calculations of protein oxidation during exposure, the subjects were asked to collect urine for 24 h beginning the morning of, and prior to, the rest and exercise in the environmental chamber. The urine was subsequently assayed for its urea nitrogen concentration (Sigma Kit 640, Sigma Chemicals Co., MO, USA).

### 3.4.4 Temperature measurements

The following were measured with an automated data acquisition system and averaged each minute: rectal temperature (Pharmaseal® 400 Series Thermistor, Baxter Healthcare Corporation, California), mean skin temperature, and mean skin heat flow using a 12-point area-weighted system as described elsewhere (Vallerand et al., 1989). For consistency in the measurement of skin temperature and heat flow, the same twelve, calibrated heat flow sensors (Concept Engineering, model FR-025-TH44033-F8-F. Connecticut) were used throughout a study.

### 3.4.5 Respiratory gas exchange measurements

Respiratory gases were monitored using a semi-automated metabolic cart system. For this purpose the subject was connected to a mouth-piece, breathing valve, and hose, which directed the expired gases to a 5 litre mixing box which was connected in series to a ventilation module which measured expired ventilation rate (VMM Ventilation Measurement Module, Interface Associates, Irvine, California). A sample line directed gases from the mixing box to oxygen (AMETEK Model S-3A11, Applied Electrochemistry, Paoli, Pennsylvania) and carbon dioxide (AMETEK Model CD-3A, Applied Electrochemistry, Paoli, Pennsylvania) analyzers. In Study 1, commercially available microcomputer based software (Vista/Turbofit Software, version 3.10, Vacumetrics Inc., Ventura, California) was used to register the data each minute and to convert the values into STPD units of oxygen consumption and carbon dioxide production. In Studies 2 and 3, custom designed computer software (DCIEM/HPP Metabolic Measurement System V1.0, Keefe and Pope, 1997) was used to register the data each minute, and to convert the values into STPD units of oxygen consumption and carbon dioxide production.

### 3.4.6 Calculation of metabolic heat production and substrate oxidation

Metabolic heat production rates ($\dot{M}$) were calculated from the respiratory gas exchange measurements of oxygen consumption, carbon dioxide production, and the respiratory exchange ratio (RER) according to Pérronet et al. (1991).

The rates of carbohydrate and fat oxidation ($CHO_{ox}$ and $FAT_{ox}$, respectively) were calculated using the non-protein oxygen consumption and the non-protein respiratory exchange ratio. Protein oxidation ($PRO_{ox}$) was assessed using the urinary urea nitrogen excretion rates (Vallerand et al., 1993). Detailed descriptions of the calculations for substrate oxidation rates are available in Vallerand et al. (1995). $PRO_{ox}$ values were not available for males therefore, gender comparisons were made using only $CHO_{ox}$ and $FAT_{ox}$.

## 4 RESULTS

This final report will focus on the effect of gender on components of the ST model with the purpose of determining whether there is a need for gender specific factors/adjustments in the ST model. For other detailed results for each Study, please refer to the relevant sections of Annexes A,B and C.

## 4.1 Study 1: Quantification Of Carbohydrate And Lipid Oxidation Rates In Shivering Females

### 4.1.1 Cold water immersion

#### 4.1.1.1 Subject characteristics

**Table 1** summarizes the subject characteristics in each group. Note that the SA/VOL [surface area to volume (mass/density)] ratio is tabled instead of the conventional measure of SA/mass since the ratio is intended to reflect the exposure area relative to overall body size. Gender differences when using data from all subjects (group ALL) were observed for height and body fatness. Body mass, SA, and SA/VOL were significantly different between males and females of comparable body fatness that were immersed for 60 min (subgroup S60). Gender differences were found in all subject characteristics except for age in those subjects that were immersed for 90 min (subgroup S90). It is noteworthy that while SA/VOL was significantly different between genders in S60 and S90, SA/mass (not shown) was found to be significantly different in all groups.

#### 4.1.1.2 Core temperature and metabolic heat production

**Figure 1** shows the mean $T_{re}$ profile of all the female subjects immersed in the cold water. All but three subjects responded with an initial transient rise in $T_{re}$ and none reached the cut-off criterion of 35°C. Two subjects requested early withdrawal after 60 min of immersion. Net decreases in $T_{re}$ ranged from 0.27 to 1.47°C. **Figure 2** shows the mean metabolic heat production of all female subjects. Its value increased to approximately 3.2 times the resting metabolic rate after 30 min of immersion. Also shown in **Figures 1 and 2** are the mean responses of all the male subjects (see Martineau and Jacobs (1989) for more details).

**Table 2** summarizes all the subjects' thermoregulatory responses to cold water immersion grouped by gender. The only significant gender difference found was in the rate of change of $T_{re}$. No significant differences were found in M when further normalized against lean body mass (LBM) or SA. Nor was there any gender difference in the resting metabolic rates (48.5 ± 14.1 and 43.7 ± 6.8 $W \cdot m^{-2}$ for males and females, respectively) used to determine $M_{shiv}$.

According to Tikuisis and Giesbrecht (31), $M_{shiv}$ is inversely proportional to the square root of %BF for given core and mean skin temperatures; i.e.:

$$M_{shiv} = \frac{A}{\sqrt{\%BF}} \qquad (4)$$

Therefore, the proportionality constant 'A' can be estimated from the product $M_{shiv} \cdot \sqrt{\%BF}$. The value of $M_{shiv}$ was determined from the difference between the subject's metabolic rates measured at the lowest common $T_{re}$ and at rest. No difference was found in the estimated value of A suggesting that the shivering drive is not different between genders under mild hypothermic strain during cold water immersion.

In order to determine the effect of menstrual cycle phase, the above analysis was repeated with only the female subjects subgrouped according to their menstrual cycle phase [follicular (n = 4) and luteal (n = 7)]. There were no differences between these subgroups in any of their physical characteristics or thermoregulatory responses listed in Tables 1 and 2. The only difference found was in their immediate pre-immersion $T_{re}$ [36.85 ± 0.07 and 37.12 ± 0.15°C (p = 0.01) for the females in their follicular and luteal phases, respectively], yet this had no impact on their subsequent thermoregulatory responses.

The regression of the pooled data of $\Delta T_{re}/\Delta t$ against the physiological variables listed in Table 1 yielded SA/VOL as the only significant independent variable [$\Delta T_{re}/\Delta t$ ($°C \cdot h^{-1}$) = 3.36 - 0.144·SA/VOL; p = 0.04, r = 0.42]. The regression of $M_{shiv}$ (using data

at the lowest measured common value of $T_{re}$ = 36.8°C) yielded BF as the only significant independent variable [$M_{shiv}$ ($W \cdot m^{-2}$) = 160.8 - 3.49·%BF; p = 0.00, r = 0.56].

**Table 3** summarizes the responses by gender of the subjects selected to have similar body fatness and a completed immersion time of 60 min (subgroup S60). No significant gender differences were found in the body cooling and metabolic rates.

**Table 4** summarizes the responses by gender of the subjects who completed 90 min of cold water immersion (subgroup S90). The only significant gender difference found was in the absolute total heat production (HP). However, when normalized against total body mass, lean body mass, or body surface area, no significant differences in HP emerged. If these data were further confined to subjects of similar body fatness (using the selection criterion in S60 resulting in 5 subjects per gender group), there were also no differences in any of the HP variables including the absolute value.

#### 4.1.1.3 Substrate oxidation

There was no significant gender difference in substrate utilization in those subjects who completed 90 min of immersion (S90). To examine whether the pattern of substrate utilization was different over this period of immersion, the relative contributions of $\%FAT_{ox}$ and $\%CHO_{ox}$ were determined during 30 min intervals. Males showed a significant increase in $\%FAT_{ox}$ (54% to 66%) and a corresponding significant decrease in $\%CHO_{ox}$ (46% to 34%) from 30 to 60 min, which leveled off during the last half hour of immersion. Females, on the other hand, showed no significant changes over time ($\%FAT_{ox}$ and $\%CHO_{ox}$ were 64% and 36%, respectively).

Subgroup S90 was further segregated ($S90_{REL}$) to only involve subjects with similar relative intensities of shivering heat production compared to their maximal aerobic power. No significant gender difference in contributions of substrate utilization was found among the subjects in this subgroup [5 females and 5 males] whose relative shivering intensities were 31 ± 2 and 29 ± 6% of $\dot{V}O_{2max}$, respectively. Yet, the same

shift in substrate utilization with time from carbohydrates to fat occurred in males in $S90_{REL}$ (**Figure 3**) as in S90.

#### 4.1.1.4 Muscle glycogen content

Muscle glycogen samples were obtained from all but two female subjects in the S90 subgroup. Concentrations decreased significantly between the pre- and post-immersion samples (493 ± 132 to 382 ± 76 mmol glucose·kg$^{-1}$). When compared to the corresponding decrease in the male sample concentrations (405 ± 64 to 312 ± 42 mmol glucose·kg$^{-1}$), no main effect of gender was found.

#### 4.1.1.5 Blood metabolites

Blood metabolite concentrations before and after cold water immersion are provided in **Table 5**. In the 4 to 5 females in S90 for whom pre- and post-immersion samples were obtained, cold water immersion did not affect levels of β-OH, epinephrine, and glucagon, however, significant increases were observed in all other metabolite and hormone concentrations. Insulin increased from 7.3 ± 2 to 10.3 ±3 μU·mL$^{-1}$, and norepinephrine from 225 ± 28 to 1017 ± 478 pg·mL$^{-1}$. The decrease in plasma volume (19.3 ± 4.7%) may be partly responsible for the increased blood metabolite levels of glucose and norepinephrine. However, the changes in the concentrations of FFA (120%), glycerol (255%), insulin (41%), and lactate (163%) were too large to be attributed only to the change in hemoconcentration. Blood analyses for the males were limited to FFA, β-OH, glycerol, glucose, and lactate (**Table 5**). All but glucose concentration increased significantly in females during the immersion. When these data were compared to those in males who completed 90 min of immersion, gender differences were seen in the responses of FFA ($p<0.05$), glycerol ($p<0.02$) and glucose ($p<0.008$). There was no significant gender difference in the magnitude of plasma volume change.

### 4.1.2 Effect of dietary manipulation

In the second part of this Study 1, eight females underwent an exercise and diet regime designed to either increase (H) or decrease (L) muscle glycogen stores before cold water immersion. These data were compared to those of eight males who had completed the same protocol. The male data were previously published (Martineau and Jacobs, 1989) but are reported again in this report for the sake of comparison. There were no statistical comparisons performed between genders, only between trials C, H and L within a gender. Observations regarding how each gender responded to the different trials follows. Subject characteristics are provided in **Table 6.**

#### 4.1.2.1 Muscle Glycogen Content

While pre-immersion muscle glycogen concentrations were significantly altered in males, females showed no significant effect of the exercise regime and dietary manipulation on muscle glycogen concentration (**Table 7**). In males, muscle glycogen concentrations in L were 60% of C which were 74% of H. Furthermore, in males, muscle glycogen decreased during exposure in H and C but not L. There was no change in muscle glycogen content during cold water immersion in females.

#### 4.1.2.2 Blood metabolites.

The low CHO treatment resulted in higher pre-immersion FFA, glycerol and ß-OH concentrations in both genders (**Table 8**) indicating a greater reliance on fat oxidation to meet metabolic demands. In males only, there were also lower pre-immersion glucose levels during L compared to H and C. There was no significant effect of trial on blood metabolites during immersion in females, however in males, the L and H cold water immersions resulted in increased blood lactate levels of a similar magnitude (113±13%).

#### 4.1.2.3 Core temperature and immersion duration

There was no effect of trial on total change in core temperature in males or females. However, in males only, the rate of decrease in $T_{re}$ (**Table 7**) was lower during L (1.5±.2°C•h$^{-1}$) compared to H (1.1±0.2°C•h$^{-1}$) or C (1.3±0.2°C•h$^{-1}$). Also in males, $T_{re}$

tended to decrease earlier during L compared to H and C ($p<0.0.08$). There was no effect of trial on immersion time in females, but in males, the immersion time was shorter during L (52±8 min) compared to C (63±9 min) or H (64±9min) (**Table 7**).

#### 4.1.2.4 Metabolic rate

There was no significant inter-trial difference in total metabolic heat production for males or females (**Table 7**). However, in males, the metabolic heat production was lower during the first 30 min of the L trial (15.1±1.5 $kJ \cdot min^{-1}$) compared to C and H that were similar (17.4±0.4$kJ \cdot min^{-1}$).

#### 4.1.2.5 Substrate utilization

In the male study, substrate oxidation data had been analyzed with the assumption that protein oxidation was zero. Therefore, for the sake of a more accurate gender comparison, the substrate oxidation in the female study was re-analyzed, assuming no protein oxidation. Group means for FATox and CHOox are provided in **Table 7.** In females, total CHOox was lower and total FATox was higher during the 90 min immersion, in L compared to H ($p<0.05$) but not C. In males, total FATox was greater ($p<0.003$) and CHOox was lower ($p<0.005$) during L than during C or H.

## 4.2 Study 2: Effects of Exercise When Cold-Stressed on Substrate Utilization

### 4.2.1 Subject characteristics

Subject characteristics are provided in **Table 9.** Twelve males participated in the previous study, six in each of the groups MI and LI.

### 4.2.2 Muscle glycogen content

Muscle glycogen contents pre-and post-exercise for males and females are provided in **Table 10**. In males, muscle glycogen concentrations decreased significantly to the same extent (22%) in MI at 9°C and 21°C. In LI, there was a significant decrease

only at 9°C. In contrast, females showed no effect of temperature on muscle glycogen content which decreased significantly in MI but not LI.

### 4.2.3 Metabolic rate

Metabolic rate response to the 30 min rest and 30 min exercise during both intensities at both temperatures are provided in **Table 11**. In females, during rest, $\dot{M}$ was significantly higher ($p<0.005$) at 9°C than at 21°C. During exercise, $\dot{M}$ was greater at 9°C compared to 21°C ($p<0.02$) in LI but not MI. However, $\dot{M}$ was only 13.4% greater at 9°C than 21°C in LI. There was no significant difference for the MI group between $\dot{M}$ at 21°C and 9°C.

In males, there was no significant effect of temperature on oxygen uptake or RER, however this must be seen in light of the protocol used to determine oxygen uptake. In the male study, metabolic samples were taken during the final 1 – 2 min of every 10 min interval rather than continuously, as in the females. It has been reported that non-specific arousal inhibits shivering (Shimada and Stitt, 1983) and that at 9°C, shivering is intermittent. It is possible that the arousal associated with insertion of the mouthpiece and application of nose clips provided sufficient stimuli to suppress shivering for the short period that $\dot{V}O_2$ was measured.

### 4.2.4 Substrate oxidation

Neither exercise intensity nor ambient temperature significantly affected the type of substrate used during each exposure. When substrates were grouped together, substrate oxidation rates were greater ($p<0.005$) at 9°C ($31\pm25$ $J\cdot s^{-1}$) than at 21°C ( $26\pm16$ $J\cdot s^{-1}$) during the rest period prior to exercise. During exercise, there was an effect of ambient temperature on substrate oxidation in LI but not MI ($p<0.02$). Substrate utilization was greater in LI at 9°C ($116\pm92$ $J\cdot s^{-1}$) than at 21°C ($103\pm78$ $J\cdot s^{-1}$). There was no difference in the relative contributions of each of the substrates to fueling $\dot{M}$ in either group during exercise at either 9°C or 21°C. Averaged over both trials, $CHO_{0x}$

contributed 62±11%, $FAT_{ox}$ contributed 36±11%, and $PRO_{ox}$ contributed 2.4±0.8% to fueling $\dot{M}$. Unfortunately, no substrate oxidation data were available for males.

## 4.3 Study 3: Relative Intensity Of Muscular Contraction During Shivering

### 4.3.1 Subject characteristics

The characteristics of fifteen females and ten males exposed to 10°C ambient air in a supine position for 2 hours are provided in **Table 12**. Statistical analysis indicated gender differences in age ($p<0.005$), height ($p<0.0001$), mass ($p<0.002$), body fat composition ($p<0.0001$), BSA ($p<0.009$) and SA/VOL ($p<0.03$).

### 4.3.2 Shivering onset

As described earlier, shivering onset was determined as the half time of shivering response. These data are presented in **Figure 4**. Males exhibited a lower $t_{1/2}$ (indicating a faster rate to steady state), than females in FE (23.4±4.2 vs. 45±7.7 min) and AB (5.06±1.27 vs. 13.96± 2.02 min), but a higher value in PE (19.78±1.61 vs. 10.92 ± 1.53 min), BB (47.95 ± 5.84 vs. 30.89 ± 3.45 min) and BR (67.88 ± 15.4 vs. 35.37 ± 4.77 min). There was no significant gender difference in GA.

### 4.3.3 Shivering intensity

In females, each of the peripheral muscles contributed less than 6% to the total EMG compared to the central muscles, PE and AB, which contributed 48±28 and 36±27%, respectively. There was no significant difference between PE and AB, however, each of these muscles contributed significantly more to total EMG than any of the peripheral muscles ($p<0.001$). There was no significant change over time of exposure in the %contribution of a given muscle to total EMG activity.

To explore the gender differences in the contributions of different body regions to shivering, data was grouped according to region (**Figure 5**). The legs (FE + GA) contributed more ($p<0.05$) to shivering in males than females (mean±SE: 26±2.17 and 8.8±1.5, respectively). The opposite was true for the trunk muscles (PE + AB) which

contributed more ($p<0.05$) in females than males (mean±SE: 84.5±1.8 and 70.7±2.2, respectively).

No gender differences were found in whole body shivering index (SUM). When averaged over both genders, SUM increased with time from 3.5 ± 0.5% to 6.4 ± 0.8% during the first 90 min and then levelled off for the last 30 min of exposure.

### 4.3.4 Metabolic heat production

Metabolic heat production in males and females prior to and during the cold air exposure is illustrated in **Figure 6**. There was no significant gender difference in $\dot{M}$ during the exposure. $\dot{M}$ increased in both males and females for the first 90 min of exposure and then levelled off. As mentioned above, whole body shivering (SUM) followed this pattern also. One objective of this study was to relate the observed increase in muscle contraction to an increase in $\dot{M}$. **Figure 7** illustrates the relationship between the whole body index of shivering (SUM) and the heat production due to shivering ($\dot{M}$ per Kg LBM). There was a positive relationship between $\dot{M}$ and SUM in females ($r = 0.66$, slope = 4.54). The correlation coefficient for males ($r = 0.17$) is considerably weaker and may be due to differences in measurement technology at the time of the experiments. However, a much stronger correlation for males emerged if individual data at different times during the exposure were regressed instead. In this case, the correlation coefficients ranged from $r = 0.66$ to 0.97 (Bell et al. 1992). The slope of the grouped data was 3.63% MVC per $W^{-1} \cdot$Kg LBM, compared to 4.54% for the mean of the female response. Some factors that might explain the gender differences (although these measures were obtained differently and have not been established to be significant) include electrode placement, subcutaneous fat, recruitment of other muscles, and non-shivering thermogenesis.

### 4.3.5 Core temperature

The mean core temperature responses for 13 females and 10 males during 15 min intervals of the 2 h exposure are provided in **Table 13**. Data were unavailable for two female and two male subjects due to problems with the rectal thermistor. No gender

differences in core temperature response to cold exposure were found. In both genders there was a significant transient rise in $T_{re}$ during the first 45 min ($p<0.001$) which slowly decreased during the last hour. However, mean $T_{re}$ at 120 min was still greater than during pre-exposure ($p<0.001$).

# 5 DISCUSSION

The primary purpose of this project was to quantify specific factors that characterize shivering metabolism ($M_{shiv}$) in females and to determine the relevance of these factors to the mathematical modeling of survival time (*ST*) in the cold. To facilitate this study, experiments were replicates of those conducted previously on males so that direct comparisons could be made to reveal any gender differences in thermoregulation. Such comparisons are essential since the present model prediction of *ST* (Tikuisis 1995, 1997) is based on the male response to cold and is assumed applicable to females. Data from this study allows us to examine this assumption critically and to make appropriate changes, if necessary.

## 5.1 Background:

It is important to relate the type of data reported herein to the modeling objective of this study. Mathematical modeling of cold response can be categorized according to the level of thermal strain involved. Low or mild levels of cold strain are associated with a deep body or core temperature ($T_{co}$) in the range from 35.5 – 36.8°C. Data density is highest at this level since most cold exposure experiments are either not stressful enough to cause $T_{co}$ to reach 35.5°C or terminated before 35.5°C is attained. While the level of cold stress (discomfort felt by the subject) may vary considerably, thermoregulation is understood to depend primarily on the level of cold strain experienced by the subject (Benzinger 1969). In essence, $M_{shiv}$ depends primarily on changes in body temperatures and not on the cold stress *per se*. Most models of thermoregulation have been developed using data involving mild levels of cold strain (eg., Tikuisis et al. 1988).

The next level of cold strain falls outside the near thermoneutral zone described above and is considered severe to moderate pertaining to a $T_{co}$ between 30.0 and 35.5°C. Relatively few experiments are conducted that lead to core temperatures in this range. Even fewer models are calibrated with such data. The DCIEM model prediction of *ST* is an exception and has been modified to correspond to data obtained from very cold water immersions that resulted in $T_{co}$ values as low as 33.25°C (Tikuisis and Giesbrecht 1999).

The final level of cold strain is considered extreme and pertains to a $T_{co}$ below 30.0°C. Understandably, controlled data are not available for this level of cold strain. Instead, researchers must ultimately rely on well-documented case histories to calibrate model predictions of ST.

The logical first step to test the validity of a model prediction of *ST* for females is to compare their thermoregulatory responses to males under conditions where data are most abundant. This was the rationale for choosing experimental conditions involving mild cold strain. Further, the prediction of *ST* is not limited to extreme cold strain, but also involves calculations that encompass conditions from thermoneutrality through lethal hypothermia. Consequently, if differences in thermoregulatory responses between genders are found under conditions of mild cold strain, such as at the beginning of a cold exposure, then these differences will propagate and ultimately skew *ST* predictions.

The physiological variables of primary interest in this study are the inputs and outputs for *ST* predictions. The input or independent variables include age, body stature (mass and height), and body fatness. Dependent variables include body temperatures (core and skin) and shivering metabolism. Additional variables measured in this study that might impact on *ST* predictions, but are presently not considered by the model, include shivering onset rates, muscle contribution to shivering metabolism, and the type of substrate utilization. Finally, it is emphasized that the present subjects represent a normal population, although certain of these were sub-grouped to remove gender differences in key characteristics such as body fatness.

## 5.2 Study 1: Quantification Of Carbohydrate And Lipid Oxidation Rates In Shivering

This study examined the thermoregulatory responses of subjects immersed to the neck level in 18°C water for up to 90 min resulting in a mild level of cold strain. Compared to the male response, the females' core temperatures cooled at about half the rate; however, when subjects were grouped according to similar body fatness, no differences were noted. Gender differences were also absent in the metabolic rates (normalized for body surface area or mass), and unexpectedly in the percentage of fat oxidation. The former is especially important since shivering impulses can be assumed to have been the same (water immersion ensured similar skin temperatures and the rate of core cooling was not different). It is concluded, therefore, that no discernible gender difference in thermoregulation exists under these conditions. That the percentage of fat oxidation was also not different suggests no gender advantage of carbohydrate sparing, which theoretically might extend ST. In conclusion, there is no requirement to alter the model prediction of ST provided that the model adequately accounts for body stature and fatness. That is, differences in predicted *ST* during cold water immersion are related to physiological variables, not gender *per se*.

The second part of the study involved an exercise regime and dietary manipulation to alter the pre-immersion muscle glycogen levels. While these levels changed significantly in males, no change occurred in the female subjects although changes in blood metabolites indicated a greater reliance on fat oxidation during exercise in both genders. That the metabolic rate was initially lower (during the first 30 min of immersion) and the consequential rate of deep body cooling was higher in males compromised by lower initial muscle glycogen levels has modeling implications. One strategy is to suppress initial shivering activity in glycogen-depleted males; however, the amount of suppression cannot be generalized from the present data. That is, only one point can be extracted from the results, that being a suppression of about 13% in the initial metabolic rate related to a decrease of about 40% in muscle glycogen levels. With regard to females, the finding of no change in their metabolic rate between trials is consistent with no change in their muscle glycogen levels. A complimentary model strategy is to reduce the amount of glycogen stores for glycogen-depleted males. The effect of this would be a shorter shivering endurance time based on the glycogen-dependence algorithm developed by Wissler (1985) and incorporated into the *ST* model.

However, the results of the present investigation are inconclusive in this regard since subjects were not exposed to the cold sufficiently long to measure shivering endurance capacity. Further, any alterations regarding female responses are even less certain. Thus, the present recommendation from this study is to apply the *ST* model assuming normal glycogen levels and to interpret the predictions liberally if it is known that pre-exposure muscle glycogen is depleted, especially in males.

## 5.3 Study 2: Effects of Exercise When Cold-Stressed on Substrate Utilization

This study examined the muscle glycogen levels and thermoregulatory responses of subjects that were initially sedentary for 30 min and then exercised for the following 30 min at one of two intensities (low and moderate) and at one of two air temperatures (9 and 21°C). The only significant differences observed were during the low intensity exercise trials where the muscle glycogen levels of males and metabolic rates of females increased during the 9°C air exposure compared to the warmer condition (metabolic values for the males were not available for analysis). The absence of a decrease in muscle glycogen levels of females during the LI cold exposure suggests that the 13% increase in metabolic rate (due to shivering) was insufficient to elicit a significant muscle glycogen utilization rate. Another possible explanation is that in females, the replenishment rate of muscle glycogen was sufficiently high to match its utilization rate. Nicklas et al. (1989) found that the magnitude of glycogen replenishment was greater during the luteal phase of the menstrual cycle than the follicular phase. While the menstrual cycle phase was not controlled in the present study, about 4-5 experiment trials were conducted in LI during the luteal phase when glycogen synthesis is elevated thus contributing to an overall lack of change in the females. Whatever the mechanism involved, the maintenance of normal glycogen levels in females was at least consistent with the results of Study 1.

From a modeling perspective, exercise can be viewed as an auxiliary source of heat production that suppresses the shivering drive (Hong and Nadel 1979). The current procedure in the ST model is to predict the suppression by the natural exponent of –2.78 x relative exercise intensity which, for example, leads to 50 and 75% suppressions of $M_{shiv}$ for relative exercise intensities of 25 and 50%, respectively. It is well known that

exercise endurance is limited disproportionally to the level of relative exercise intensity; i.e., endurance falls rapidly with increasing intensity (Gleser and Vogel 1973). This characteristic has been adopted by Wissler (1985) as the basis of shivering fatigue except that relative shivering intensity is gauged by maximum shivering intensity (typically about five times resting metabolism). Thus, shivering fatigue is predicted to occur rapidly if an individual shivers at close to their maximum intensity; yet, if exercising at this level in absolute terms, the same individual could endure the effort almost indefinitely. The recommended modeling strategy to deal with exercise during cold exposure is to calculate the 'endurance' expenditure separately for exercise and shivering, and to combine these expenditures until exhaustion is predicted at which point the metabolic rate must decrease. Unfortunately, data on shivering fatigue are presently nonexistent to test this strategy. With regard to gender differences, it is not known from the male data whether an increase in shivering metabolism during the low intensity exercise trial at 9°C was higher than that observed for females. However, considering that body fatness levels were lower in males, it would be reasonable to assume that their shivering intensities were higher and therefore consistent with the measured decrease in their muscle glycogen levels. Predicted survival times would be reduced accordingly. The above strategy to deal with exercise during cold exposure would support this expectation.

## 5.4 Study 3: Relative Intensity Of Muscular Contraction During Shivering

This study examined the thermoregulatory responses of subjects exposed to 10°C air for 2 h resulting in a mild level of cold strain. In this case, no gender differences emerged in the core cooling rate or shivering metabolism. Instead, differences were found in the shivering onset rates of various muscles. Males were observed to have a sooner shivering onset in the thigh and abdominal regions, but a later onset in the arms and upper torso. While not presently considered in the prediction model of *ST*, onset differences are probably of secondary importance as evidenced by the lack of gender differences in the more general thermoregulatory responses. Nevertheless, such differences should be considered for completeness in future models.

Another gender difference that emerged in this study was the percentage of muscle contribution to overall shivering metabolism. Males exhibited higher contributions from the leg muscles than females whereas the opposite occurred in the torso region (differences were not significant in the arms). It is uncertain how these differences might have affected the observed overall body cooling rates. Theoretically at least, the legs are more susceptible to heat loss than the torso due to a higher surface area to volume ratio. Hence, increased heat production in the legs is not efficient in terms of heat debt management. Yet, the data did not indicate gender differences in overall body cooling rates. It is possible that differences in fat distribution between males and females had a canceling effect. That is, relatively higher subcutaneous fat levels in the male abdominal region and in the female thigh region provide greater insulation to these respective regions of relatively lower heat production such that overall heat production was more evenly retained. Such subtleties should be recognized in more advanced models of thermoregulation, but are not deemed essential in current model predictions of ST. Indeed, current predictions rely mostly on the cooling of the torso and the present results confirm that in both genders most of the shivering heat production (> 70%) occurs in the torso and that its onset is relatively rapid compared to the other regions of the body.

## 5.5 Summary:

The series of studies conducted herein was purposely limited to conditions of mild cold strain as a necessary first step in the examination of the suitability of current *ST* prediction models for females. Although certain of the findings indicated gender differences in the response to cold, these responses (muscle glycogen levels and regional shivering onsets and contributions) are considered secondary in importance to the primary responses of thermoregulation such as shivering heat production and rate of deep body cooling. With regard to these latter variables, no gender differences were found if physiological variables such as stature and body fatness were taken into account. In fact, a recent report by Gonzalez et al. (1998) endorsed the use of the shivering heat production equation regressed by Tikuisis et al. (1991) in the cold air study on males used herein as the basis for Study 3. Body fatness is an essential factor in this equation and the analysis by Gonzalez et al. supports its application to females. Thus, in the absence of

any contravening evidence, current prediction models of *ST* can be considered gender-invariant if physiological characteristics are adequately applied.

## 5.6 Summary of hypothesis

**Study 1:** *"The relative increase in metabolic heat production ( $\dot{M}$ ) caused by cold water immersion will be less in females than what has previously been reported for males."* Rejected.

*"Compared to previous reports for males, a smaller proportion of the increase in $\dot{M}$ will be due to increases in carbohydrate oxidation."* Rejected.

*"Unlike what has been reported for males, muscle glycogen depletion of large skeletal muscle groups will not impair body temperature regulation during cold water immersion."* This hypothesis could not be tested.

**Study 2:** *"$\dot{M}$ will increase to a greater extent when light exercise is performed during cold air exposure than when performing the same exercise at a comfortable temperature."* Accepted.

*" Contrasting with previous reports for male subjects, this increase will not be associated with any greater muscle glycogen utilization than is the case when the same exercise is performed at a comfortable temperature."* Accepted.

**Study 3:** *This study is descriptive thus no experimental hypotheses were postulated a priori.*

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# 7 TABLES.

Table 1. Subject characteristics (mean ± SD); * indicates a significant gender difference within a group. ALL, S60, and S90 refer to groups containing all subjects, those of similar body fatness that were immersed for 60 min, and those that were immersed for 90 min.

| Group | Gender | n | Age (yr) | Mass (kg) | Height (cm) | SA ($m^2$) | SA/VOL ($m^{-1}$) | BF (%) |
|---|---|---|---|---|---|---|---|---|
| ALL | male | 14 | 25.5 ± 4.3 | 72.3 ± 11.1 | 176 ± 11* | 1.89 ± 0.19 | 28.3 ± 1.8 | 10.6 ± 4.4* |
| | female | 11 | 24.4 ± 6.3 | 65.0 ± 7.8 | 167 ± 8 | 1.80 ± 0.13 | 29.1 ± 1.6 | 22.4 ± 5.9 |
| S60 | male | 5 | 25.0 ± 3.9 | 81.4 ± 8.9* | 179 ± 8 | 2.02 ± 0.14* | 26.5 ± 1.4* | 15.3 ± 2.7 |
| | female | 6 | 26.5 ± 6.8 | 67.1 ± 4.1 | 171 ± 5 | 1.84 ± 0.06 | 29.0 ± 1.0 | 18.3 ± 3.0 |
| S90 | male | 7 | 25.9 ± 4.6 | 78.9 ± 8.4* | 179 ± 7* | 1.99 ± 0.13* | 27.1 ± 1.5* | 12.7 ± 5.2* |
| | female | 9 | 24.0 ± 6.0 | 66.5 ± 7.3 | 170 ± 6 | 1.82 ± 0.11 | 28.8 ± 1.5 | 23.0 ± 6.3 |

Table 2. Responses of all subjects (group ALL in Table 1) during immersion in 18°C water (mean ± SD; * indicates a significant gender difference). $\Delta t$ and $\Delta T_{re}$ are the duration of and decrease in $T_{re}$ during the immersion. M and $M_{shiv}$ are the total and shivering component of the metabolic heat production at a $T_{re}$ value of 36.8°C. $FAT_{ox}$ is the contribution of fat oxidation towards total heat production and A is the proportionality constant of $M_{shiv} \cdot \sqrt{\%BF}$ (Eq. 4).

| **Gender** | **$\Delta t$** (min) | **$\Delta T_{re}$** (°C) | **$\Delta T_{re}/\Delta t$** ($°C \cdot h^{-1}$) | **M** ($W \cdot m^{-2}$) | **$M_{shiv}$** ($W \cdot m^{-2}$) | **$FAT_{ox}$** (%) | **A** |
|---|---|---|---|---|---|---|---|
| male | 66.1 ± 27.8 | 0.86 ± 0.34 | 1.01 ± 0.67* | 164 ± 57 | 115 ± 60 | 48.7 ± 17.1 | 342 ± 132 |
| female | 83.6 ± 11.8 | 0.65 ± 0.45 | 0.47 ± 0.32 | 137 ± 33 | 94 ± 34 | 58.6 ± 11.4 | 425 ± 124 |

Table 3. Responses (mean ± SD) of subjects of similar body fatness (subgroup S60; Table 1) during immersion in 18°C water over 60 min. $\dot{V}O_2$ represents the mean rate of oxygen consumption.

| **Gender** | **$T_{re}$ @ t = 0** (°C) | **$T_{re}$ @ 1h** (°C) | **$\Delta T_{re}/\Delta t$** ($°C \cdot h^{-1}$) | $\dot{V}O_2$ ($L \cdot min^{-1}$) |
|---|---|---|---|---|
| male | 37.02 ± 0.39 | 36.60 ± 0.39 | 0.42 ± 0.49 | 0.85 ± 0.13 |
| female | 37.04 ± 0.19 | 36.74 ± 0.26 | 0.31 ± 0.26 | 0.72 ± 0.15 |

Table 4. Responses (mean ± SD; * indicates a significant gender difference) of subjects who completed 90 min of immersion in 18°C water (subgroup S90; Table 1). HP is the total heat production and $FAT_{ox}$ is the contribution of fat oxidation towards HP.

| **Gender** | **$\Delta T_{re}/\Delta t$** ($°C \cdot h^{-1}$) | **HP** (kJ) | **HP** ($kJ \cdot kg^{-1}$) | **HP** ($kJ \cdot kg^{-1}_{LBM}$) | **HP** ($kJ \cdot m^{-2}$) | **$FAT_{ox}$** (%) |
|---|---|---|---|---|---|---|
| male | 0.50 ± 0.29 | 1732 ± 407* | 22.5 ± 7.1 | 25.5 ± 6.7 | 880 ± 247 | 61.4 ± 13.4 |
| female | 0.45 ± 0.33 | 1257 ± 247 | 19.0 ± 4.0 | 24.6 ± 3.8 | 689 ± 134 | 60.4 ± 11.5 |

Table 5. Gender response comparisons (mean ± SD; √ and * indicate a significant gender difference and a main effect of time, respectively, between pre and post cold water immersion samples) in blood metabolites [in $\mu mol \cdot L^{-1}$ (μM) or $mmol \cdot L^{-1}$ (mM)] of subjects who completed 90 min of immersion (subgroup S90; Table 1).

| **Gender** | **FFA** (μM)√ | | **β-OH** (μM) | | **Glycerol** (mM) √ | | **Glucose** (mM) √ | | **Lactate** (mM) | | **PVC** (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pre | Post* | Pre | Post* | Pre | Post* | Pre | Post | Pre | Post* | |
| Males (n = 7) | 553 ± 200 | 753 ± 265 | 156 ± 83 | 237 ± 172 | .093 ± .062 | .144 ± .059 | 4.99 ± .16 | 4.81 ± .39 | .94[1] ± .36 | 1.59 ± .45 | -19.3 ± 4.7 |
| Females (n = 5) | 393[2] ± 182 | 863 ± 202 | 213 ± 132 | 347 ± 242 | .051 ± .012 | .181 ± .051 | 4.52 ± .43 | 5.24 ± .69 | .77 ± .28 | 2.11 ± 1.10 | -24.0 ± 6.0 |

[1] n = 6; [2] n = 4

Table 6. Study 1 Subject characteristics for subjects who completed all three cold water immersions.

| Gender,n | Age (yrs) | Weight (Kg) | Height (cm) | % BF (%) | BSA ($m^2$) | VO2max (ml/kg/min) |
|---|---|---|---|---|---|---|
| Males, n=8 | 26 ± 2 | 69.3 ± 3.5 | 176 ± 4 | 9 ± 1 | 1.86 ± 0.07 | 48 ± 2.2 |
| Females, n=9 | 25 ± 2.3 | 64.9 ± 2.3 | 166.9 ± 2.7 | 24.2 ± 2.3 | 1.72 ± 0.04 | 38.7 ± 2.3 |

Table 7. Metabolic and temperature responses to cold water immersion in subjects immersed at 18°C after control (C), low (L), or high (H) carbohydrate diets. Values are means ± SE. * indicates significant change during a given immersion, ! and + indicate a significant difference from C or H, respectiviely.

| | Immersion | Total change | Rate of change | M | Muscle glycogen content | | CHOox | FATox |
|---|---|---|---|---|---|---|---|---|
| Trial, Gender | time | in Tre | in Tre | | (mmol glucose•Kg dry muscle$^{-1}$) | | | |
| | (min) | (°C) | (°C•h$^{-1}$) | (J•s$^{-1}$) | Pre | Post | (%M) | (%M) |
| Control | | | | | | | | |
| Males, n=8 | 63 ± 9 | 0.98 ± 0.11 | -1.3 ± 0.2 | 311.7 ± 28.3 | 406 ± 23 | 328 ± 30 * | 59.3 ± 5.8 | 40.7 ± 5.8 |
| Females, n=9 | 86.3 ± 3.5 | 0.57 ± 0.19 | 0.39 ± 0.13 | 220.4 ± 17.8 | 489 ± 128 | 403 ± 81 | 41.2 ± 3.9 | 58.8 ± 3.9 |
| High | | | | | | | | |
| Males, n=8 | 64 ± 9 | 1.025 ± 0.07 | -1.1 ± 0.2 | 301.7 ± 18.4 | 548 ± 42 ! | 476 ± 41 * | 63.8 ± 3.8 | 35.4 ± 5.1 |
| Females, n=9 | 90 ± 0.0 | 0.57 ± .15 | 0.39 ± 0.10 | 233.0 ± 28.4 | 561 ± 156 | 499 ± 137 | 42.8 ± 4.9 | 57.2 ± 4.9 |
| Low | | | | | | | | |
| Males, n=8 | 52 ± 8 ! + | 1.075 ± 0.09 | -1.5 ± .2 ! + | 282.3 ± 33.7 | 247 ± 15 ! | 219 ± 15 | 30.6 ± 6.4 ! + | 69.4 ± 6.4 ! + |
| Females, n=9 | 90 ± 0.0 | 0.76 ± 0.19 | 0.52 ± 0.13 | 200.8 ± 20.5 | 460 ± 74 | 376 ±79 | 30.5 ± 5.6 + | 69.5 ± 5.6 + |
| | | | | | | | | |

Table 8. Blood metabolite responses to cold water immersion during Control, Low and High muscle glycogen conditions. Values are means ± SE. * indicates significant difference from Pre-immersion ($p<0.05$), + indicates difference from Control and High, ($p<0.05$).

| Metabolite | Sample | Males | | | Females | | |
|---|---|---|---|---|---|---|---|
| | | Control | High | Low | Control | High | Low |
| Glucose, mM | Pre | 4.9 ± 0.2 | 5.2 ± 0.1 | 4.7 ± 0.2 + | 4.52 ± 0.19 | 4.42 ± 0.23 | 4.58 ± .112 |
| | Post | 3.9 ± 0.1 | 3.9 ± 0.2 | 3.9 ± 0.2 | 5.24 ± 0.31 | 5.1 ± 0.23 | 5.45 ± 0.27 |
| Lactate, mM | Pre | 0.76 ± 0.06 | 0.84 ± 0.11 | 0.56 ± 0.05 | 0.77 ± 0.12 | 0.8 ± 0.15 | 0.6 ± 0.07 |
| | Post | 1.36 ± 0.11 | 1.6 ± 0.3 | 1.47 ± 0.18 * | 2.11 ± 0.49 | 2.13 ± 0.51 | 2.29 ± 0.53 |
| FFA,uM | Pre | 495 ± 97 | 459 ± 100 | 565 ± 146 + | 390 ± 90 | 360 ± 20 | 490 ± 60 + |
| | Post | 589± 104 | 475 ± 38 | 710 ± 64 | 860 ± 100 | 860 ± 140 | 990 ± 160 |
| Glycerol, uM | Pre | 68 ± 14 | 60 ± 10 | 65 ± 8 + | 51 ± 5 | 50 ± 4 | 69 ± 9 + |
| | Post | 105 ± 13 | 84 ± 11 | 144 ± 18 | 181 ± 2 | 168 ± 3 | 209 ± 38 |
| B-OH, uM | Pre | 169 ± 60 | 112 ± 30 | 554 ± 129 + | 210 ± 60 | 150 ± 40 | 400 ± 100 + |
| | Post | 162 ± 36 | 129 ± 53 | 447 ± 77 | 350 ± 110 | 300 ± 90 | 550 ± 100 |

Table 9. Subject characteristics for males and females who completed moderate intensity (MI) or light intensity (LI) exercise at 9°C or 21°C for 30 min. Values are mean ± SE.

| Gender, n | Age (yrs) | Weight (Kg) | Height (cm) | % BF (%) | W4 (watts) | Target power output (W) |
|---|---|---|---|---|---|---|
| Males | | | | | | |
| LI, n=6 | 22.3 ± 2.6 | 79.5 ± 11.9 | 150 ± 7 | 15.5 ± 6.1 | 179 ± 26 | 55 ± 6 |
| MI, n=6 | 20.3 ± 1.4 | 69.8 ± 9.1 | 172 ± 6 | 11.7 ± 4.1 | 171 ± 23 | 103 ± 14 |
| Females | | | | | | |
| LI, n=8 | 25.5 ± 5.4 | 61.3 ± 7.1 | 164 ± 6.4 | 24.4 ± 4.6 | 115 ± 32 | 34.4 ± 10 |
| MI, n=8 | 24.1 ± 6.3 | 60.5 ± 6.6 | 161 ± 11.5 | 22.4 ± 7.5 | 128 ± 31 | 76 ± 18 |

Table 10. Muscle glycogen concentration in males and females who performed light (LI) and moderate (MI) intensity exercise at 9°C and 21°C for 30 min. Values are mean ± SE. * indicates significant change during exercise.

Muscle Glycogen ( mmol•Kg dry muscle$^{-1}$)

| | 21°C | | 9°C | |
|---|---|---|---|---|
| | Pre | Post | Pre | Post |
| Males | | | | |
| LI, n=6 | 327 ± 60 | 319 ± 44 | 376 ± 50 | 289 ± 76 * |
| MI, n=6 | 384 ± 57 | 300 ± 47 * | 361 ± 69 | 297 ± 75 * |
| Females | | | | |
| LI, n=8 | 399 ± 45 | 369 ± 30 | 470 ± 73 | 441 ± 83 |
| MI, n=8 | 391 ± 123 | 285 ± 65 * | 388 ± 117 | 293 ± 102 * |

Table 11. Metabolic rate in females only who completed 30 min of rest followed by 30 min of either Moderate intensity (MI) or light intensity (LI) exercise at 21°C and 9°C. Values are mean ± SE. *indicates significant difference from 21°C.

Metabolic rate ($W \cdot m^{-2}$)

| Trial | Rest | | Exercise | |
|---|---|---|---|---|
| | 21°C | 9°C | 21°C | 9°C |
| LI | 47.6 ± 10 | 61.1 ± 16 | 184.5 ± 25 | 209.3 ± 28 * |
| MI | 45.3 ± 3.8 | 52.5 ± 9.6 | 261 ± 40 | 265.4 ± 30 |

Table 12. Subject characteristics of males and females exposed to 10°C ambient air for 2 hours.

| Name | Age (yrs) | Height (cm) | Mass (Kg) | FAT (%) | BSA ($m^2$) | SA/VOL (m^-1) |
|---|---|---|---|---|---|---|
| Males, n=10 | | | | | | |
| Mean | 35.1 | 179.4 | 77.3 | 12.6 | 1.97 | 27.4 |
| ± SD | 4.0 | 5.8 | 10.4 | 5.4 | 0.14 | 1.7 |
| Females, n=15 | | | | | | |
| Mean | 28.0 | 162.8 | 63.9 | 23.2 | 1.76 | 29.2 |
| ± SD | 6.3 | 4.8 | 8.7 | 4.8 | 0.12 | 2.0 |

Table 13. Mean core temperature response in males and females in exposed to 10°C ambient temperature for 2 h. Values are mean±SD.

Rectal Temperature (°C)

| Groups | Pre | 15 min | 30 min | 45 min | 60 min | 75 min | 90 min | 105 min | 120 min |
|---|---|---|---|---|---|---|---|---|---|
| Males | | | | | | | | | |
| Mean | 36.76 | 36.80 | 36.86 | 36.91 | 36.93 | 36.93 | 36.89 | 36.86 | 36.81 |
| ± SD | 0.21 | 0.30 | 0.27 | 0.28 | 0.30 | 0.31 | 0.32 | 0.33 | 0.36 |
| Females | | | | | | | | | |
| Mean | 36.92 | 36.96 | 37.13 | 37.23 | 37.23 | 37.19 | 37.17 | 37.14 | 37.10 |
| ± SD | 0.15 | 0.17 | 0.21 | 0.19 | 0.22 | 0.24 | 0.22 | 0.20 | 0.21 |
| | | | | | | | | | |

# 8 FIGURES

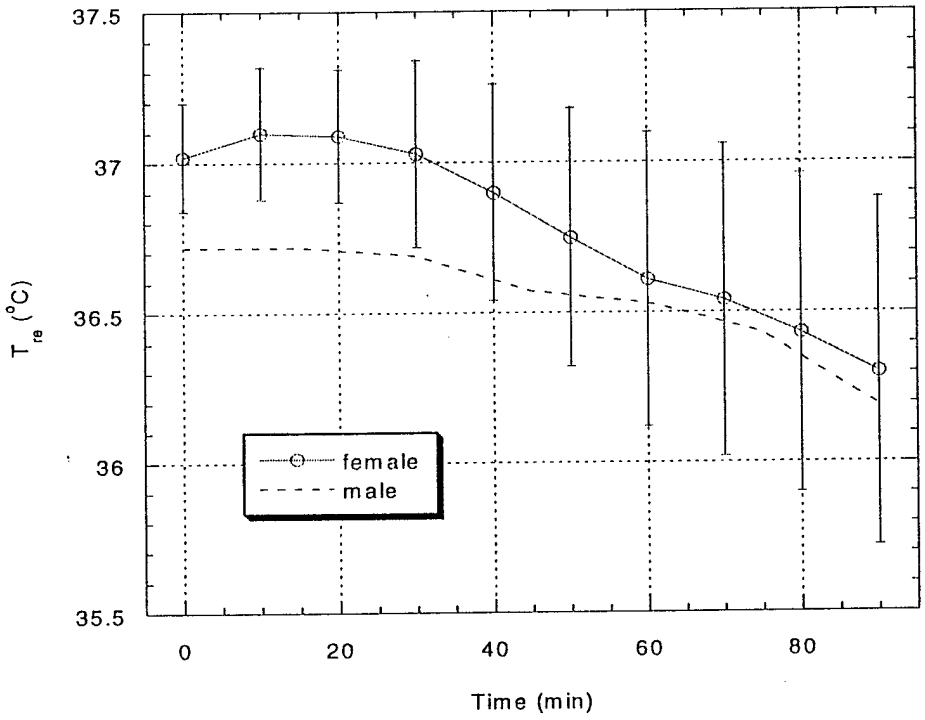


Figure 1. Mean (± SD) of the rectal temperature of females (n = 11 except after 60 min when n = 9) during neck-level immersion in 18°C water. Also shown are the mean values for males (15) where n = 14, 9, 8, and 7 for immersion times up to 30, 45, 60, and 90 min, respectively.

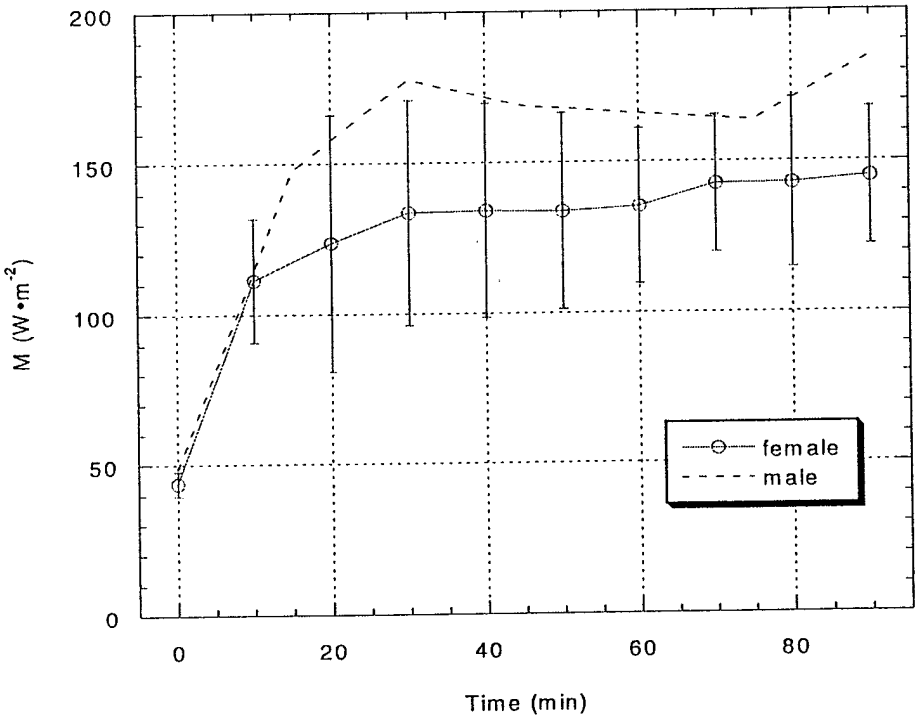


Figure 2. Mean (± SD) of the metabolic rate of females (n = 11 except after 60 min when n = 9) during neck-level immersion in 18°C water. Also shown are the mean values for males (15) where n = 14, 9, 8, and 7 for immersion times up to 30, 45, 60, and 90 min, respectively.

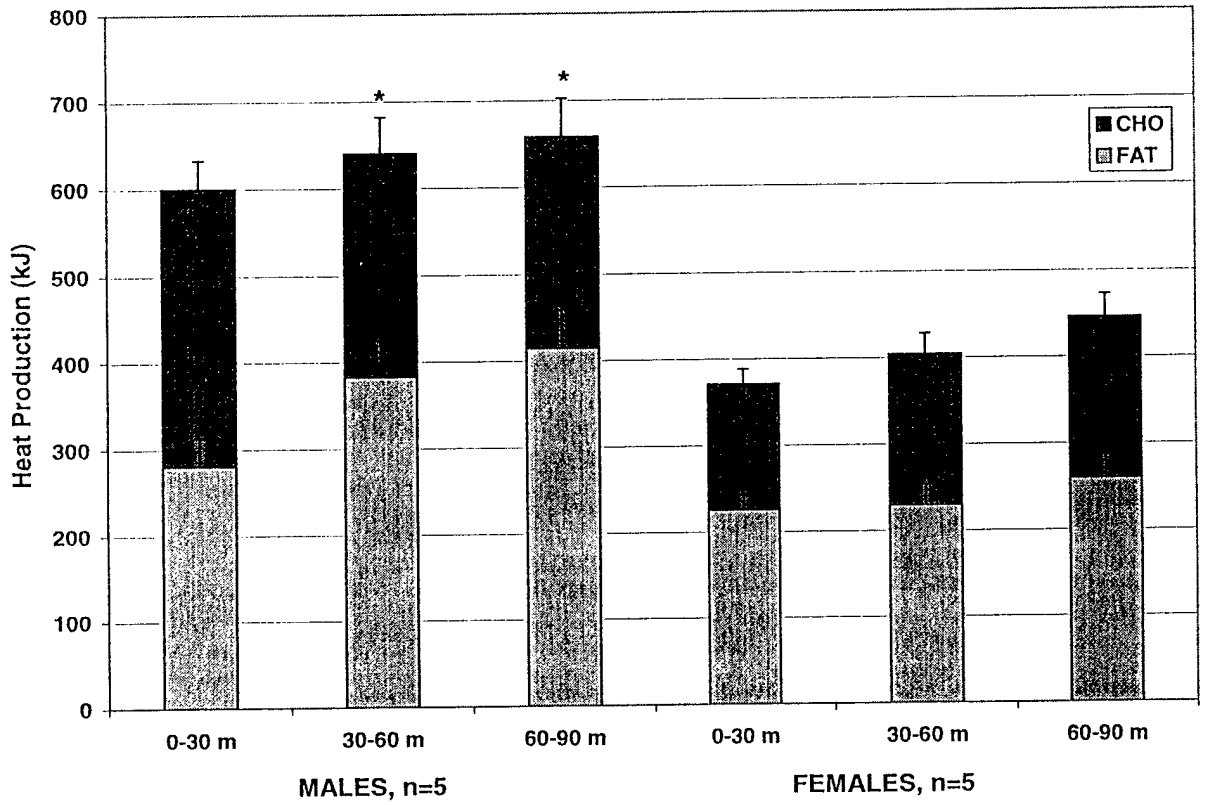


Figure 3. Mean (± SE) of the heat production due to carbohydrate and fat metabolism of males and females ($S90_{REL}$) who completed 90 min of immersion with similar intensities of shivering compared to their $\dot{V}O_2$. Differences between genders are significant; * indicates a significant increase over time among the males.

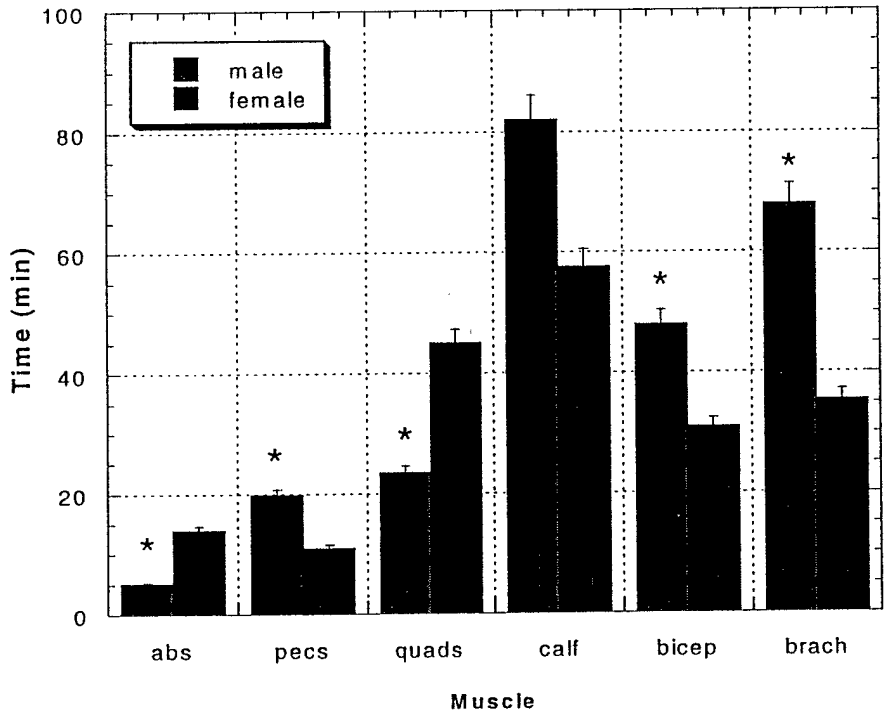


Figure 4. Onset of shivering for each muscle group in males and females exposed to10°C ambient air for 2 h. Values are means±SD. * indicates significant gender differences ($p<0.05$).

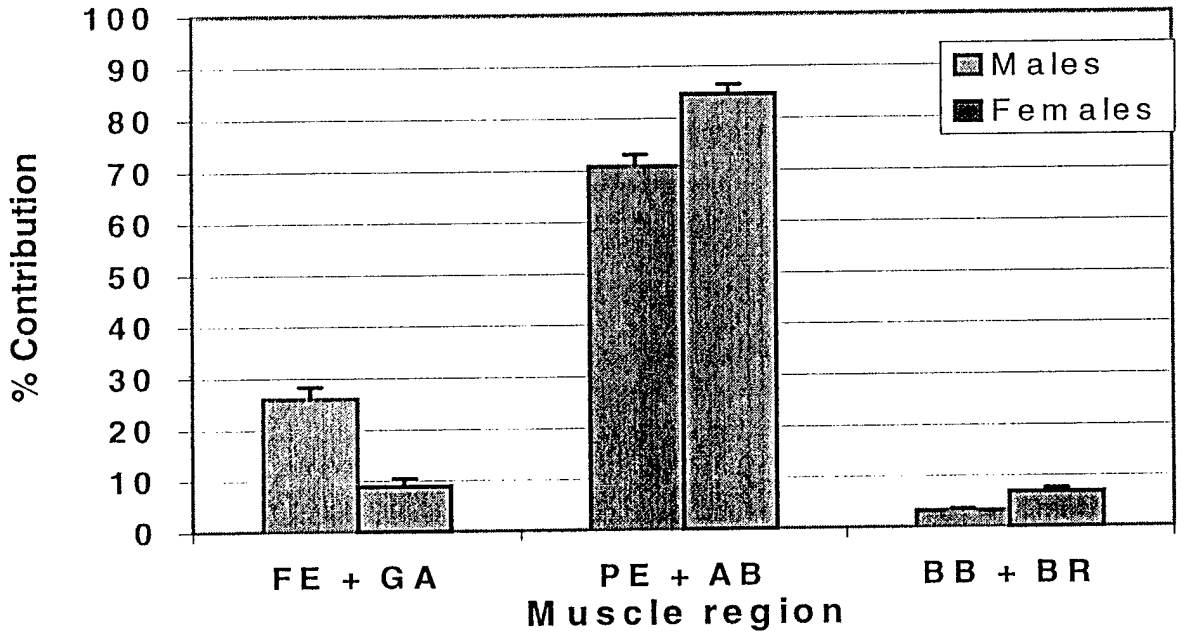


Figure 5. Electromyography (%Contribution by muscle groups shown to total EMG activity) in 10 males and 15 females before and during a 2 hour exposure to 10°C ambient air temperature in supine position. Values are means ± SE.

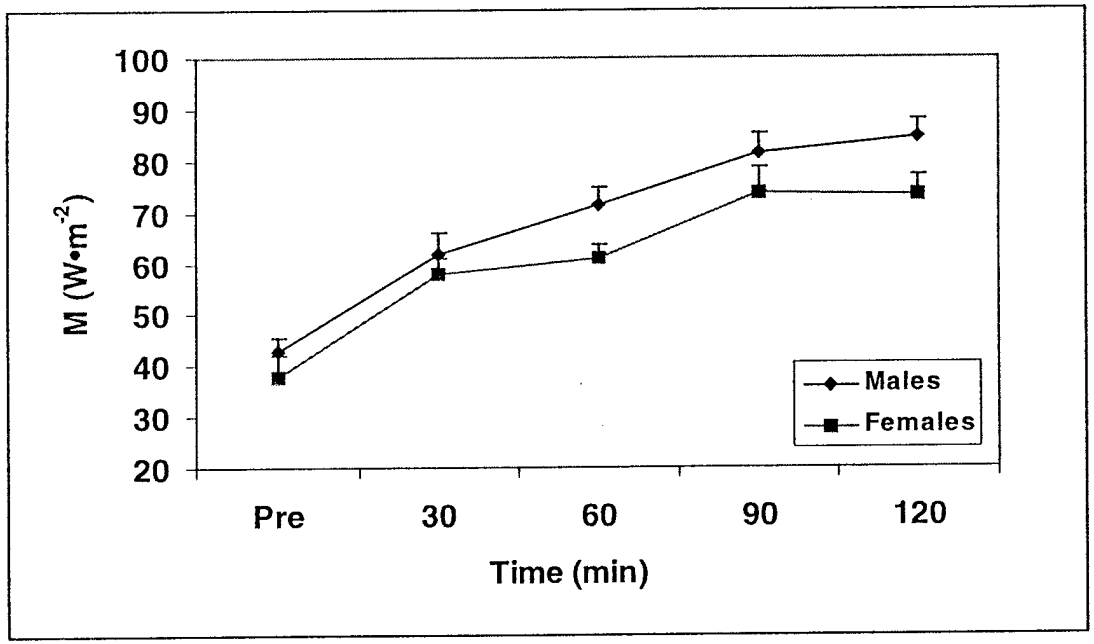


Figure 6. Metabolic heat production in males and females before and during a 2 hour exposure to 10°C ambient air temperature in supine position. Values are means ± SE.

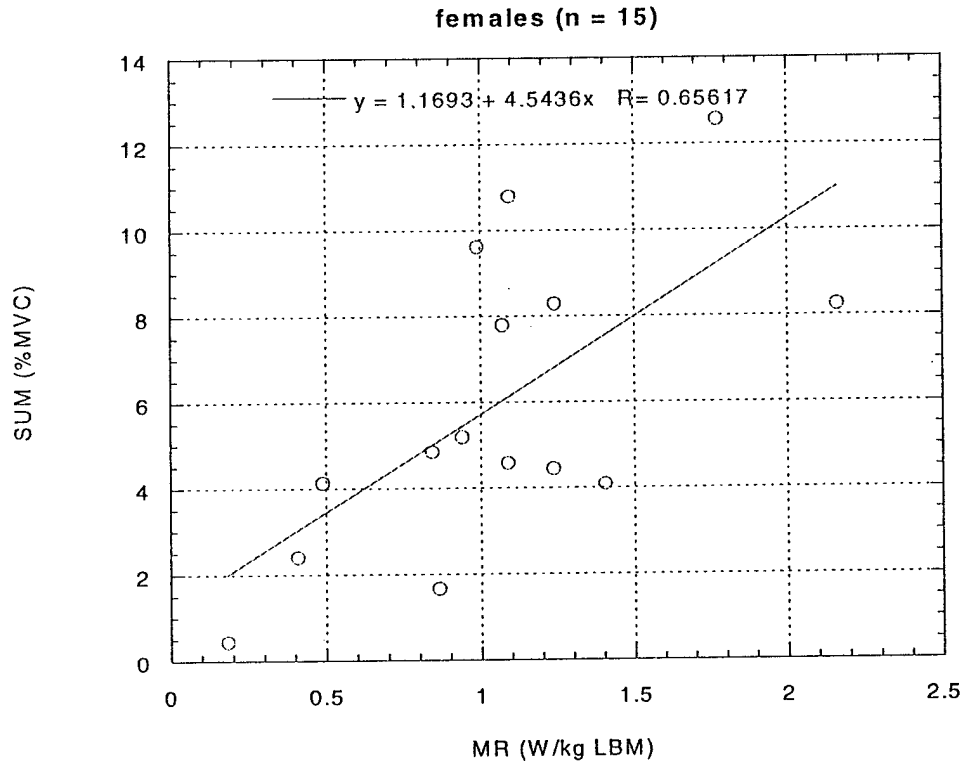


Figure 7. Correlation between whole body index of shivering (SUM) and metabolic heat production due to shivering in females before during the 2 hour exposure to 10°C air. Each point represents the mean value of a specific subject.

# ANNEX A

# ANNUAL REPORT 1

# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 0704-0188*



| 1. AGENCY USE ONLY *(Leave blank)* | 2. REPORT DATE | 3. REPORT TYPE AND DATES COVERED |
|---|---|---|
| | October 1997 | Annual (1 Oct 96 - 30 Sep 97) |

**4. TITLE AND SUBTITLE** Energy Metabolism in Cold-Stressed Females: Implications for Predictive Modeling

**5. FUNDING NUMBERS**
DAMD17-96-C-6128

**6. AUTHOR(S)**
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**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**
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North York, Ontario, Canada M3M 3B9

**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)**
Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

**10. SPONSORING/MONITORING AGENCY REPORT NUMBER**

**11. SUPPLEMENTARY NOTES**

**12a. DISTRIBUTION / AVAILABILITY STATEMENT**
Approved for public release; distribution unlimited

**12b. DISTRIBUTION CODE**

**13. ABSTRACT** *(Maximum 200*

This document is a progress report which describes the results from the first of a series of studies carried out to clarify the extent of gender-related differences in physiological responses to cold stress, and to evaluate the potential implications for survival time in the cold. Specifically, this study was designed to clarify the quantity and quality of energy substrate utilization in shivering female subjects during cold water immersion. The objectives were: to determine the magnitude of metabolic heat production during cold water immersion; to quantify the relative contributions of fat, carbohydrate and protein metabolism to fuelling metabolism; to determine if muscle glycogen is a significant energy source during shivering; to manipulate skeletal muscle glycogen availability and to evaluate the subsequent effects on metabolic rate and body temperature rgulation during cold stress. Female subjects were immersed to the neck in 18 degC water for up to 90 minutes. Their metabolic rate increased to about 3 times resting levels, similar to what what reported for males. About 40% of metabolism was fueled by carbohydrate oxidation. Muscle glycogen was a significant contributor to metabolism. The protocol was not successful in manipulating glycogen availability.

**14. SUBJECT TERMS** Defense Women's Health Research Program
Physiology, Shivering, Hypothermia, Thermoregulation, Metabolism Substrates

**15. NUMBER OF PAGES**
59

**16. PRICE CODE**

| 17. SECURITY CLASSIFICATION OF REPORT | 18. SECURITY CLASSIFICATION OF THIS PAGE | 19. SECURITY CLASSIFICATION OF ABSTRACT | 20. LIMITATION OF ABSTRACT |
|---|---|---|---|
| Unclassified | Unclassified | Unclassified | Unlimited |

# 1. TABLE OF CONTENTS

## 2. ABSTRACT

This document is a progress report which describes the results from the first of a series of studies carried out to clarify the extent of gender-related differences in physiological responses to cold stress, and to evaluate the potential implications for survival time in the cold. Specifically, this study was designed to clarify the quantity and quality of energy substrate utilization in shivering female subjects during cold water immersion. The objectives were: to determine the magnitude of metabolic heat production during cold water immersion; to quantify the relative contributions of fat, carbohydrate and protein metabolism to fueling metabolism; to determine if muscle glycogen is a significant energy source during shivering; to manipulate skeletal muscle glycogen availability and to determine the subsequent effects on metabolic rate and body temperature regulation during cold stress. Female subjects were immersed to the neck in 18°C water for up to 90 minutes. Their metabolic rate increased to about 3 times resting levels, similar to what was reported previously for male subjects. About 40% of the metabolic heat production during immersion was fueled by oxidation of carbohydrates, somewhat less than what was reported for male subjects. Also, as reported earlier for males, muscle glycogen decreased significantly during the immersion suggesting that it is a significant constituent of the carbohydrate component of metabolic heat production during shivering. The implications of manipulating glycogen availability in females could not be adequately addressed because the protocol did not result in the desired changes in muscle glycogen prior to immersion.

## 3. INTRODUCTION

Military units operate in cold air and cold water environments, and the associated training or missions can result in personnel being faced with life-threatening situations if they are ill-equipped or unprotected. As demonstrated by the recent winter crash in the Canadian Arctic of a military aircraft carrying infantry personnel, rescue can be delayed for days even when the precise location of survivors is known (de Groot, 1994). Cold water immersion hypothermia recently caused the deaths during training of US Army Rangers (Fort Benning, 1995). In light of such potential emergencies the prediction of survival time (*ST*) in the cold, defined in this document as the elapsed time until the onset of lethal hypothermia, is essential to meet the needs of Search and Rescue authorities. Such predictions are also useful in the analysis of strategic human factors demands of military operations in the cold, to prepare for contingencies of such operations, and to evaluate the potential benefits of equipment/clothing designed to protect the soldier from the cold.

An understanding of *ST* in healthy, sedentary, non-traumatized individuals is based in the following relationships. Once the protective insulation of available shelter or clothing is maximized, cold-stressed humans elevate metabolic heat production ($\dot{M}$) by shivering in an attempt to balance heat loss. Existing models of *ST* in cold air or cold water are based on observations of factors which affect $\dot{M}$ and the rate of heat loss from the body. In such models $\dot{M}$ increases as a function of temperature signals from the core and skin. When cold exposure is too severe for $\dot{M}$ to balance heat loss, *ST* is largely determined by the rate of heat loss from the body. Where there is a balance between $\dot{M}$ and heat loss, *ST* is limited by the endurance time for shivering.

The physiological factors characterizing $\dot{M}$ are relatively complex. Until about a decade ago there was very little empirically based information available in this regard for human subjects. Research has demonstrated that the relationship

between $ST$, $\dot{M}$, and heat loss is affected by the extent of the muscle mass involuntarily recruited during shivering (Bell et al., 1992), connective heat transfer during cold stress (Tikuisis et al., 1991), muscle substrate availability (Jacobs et al., 1994), the type and quantity of substrate oxidized by shivering musculature (Vallerand and Jacobs, 1989), and body composition (Tikuisis et al., 1988). Our research during the last decade has focused on such factors with the objective of generating sufficient knowledge to improve the predictive modelling of $ST$ in the cold. A brief review of this research follows.

By measuring the electrical activity of many muscle groups simultaneously during cold-induced shivering, we demonstrated that several large muscle groups are recruited and contract at relatively low intensities that are less than 20% of their maximum force generating capabilities (Bell et al., 1992). Since so many muscle groups are involved in shivering, the sum total of their contractile activities can result in a four or five-fold increase in metabolic rate, and heat production.

Much of our attention has been directed towards the substrates that are used by skeletal muscle to increase heat production during shivering. For example, Vallerand et al. (1988) administered a clinical glucose tolerance test to subjects who were sitting in either cold air or at a comfortable temperature for two hours. These data were the first to show in humans that glucose is eliminated more rapidly from the circulation during cold exposure, presumably to provide more available substrate to fuel the increase in metabolic rate. It is also noteworthy that this more rapid uptake of glucose during cold exposure occurs with lower insulin levels in the cold compared to warm temperatures.

We subsequently continued to attempt to quantify the rates of substrate oxidation of fat, carbohydrate and protein in humans during cold exposure with indirect calorimetric techniques. As one might presume, the increase in metabolic rate during shivering is caused by increases in oxidation of both fat and carbohydrate, but the relative increase in the rate of substrate oxidation caused by shivering is greatest for carbohydrates (Vallerand and Jacobs, 1989). In resting subjects exposed to either cold air or cold water, carbohydrates and fat contribute

approximately equally to heat production (Martineau and Jacobs, 1991; Vallerand and Jacobs, 1989). From a strategic point of view, this finding seems unfortunate because the body's availability of carbohydrates is quite limited compared to the abundant fat and protein stores. We were already aware of the well established positive relationship between muscle glycogen concentration and endurance exercise performance of skeletal muscle and speculated that there may be a similar detrimental effect caused by muscle glycogen depletion on another form of muscle contraction, i.e. shivering and the associated heat production.

We therefore carried out a series of studies on male subjects immersed in 18°C water. The subjects were removed from the water when their rectal temperature reached 35.5° C. Biopsies were taken from the thigh muscle before and after the immersion to evaluate the changes in glycogen as a result of the water immersion (Martineau and Jacobs, 1988). In another study muscle glycogen concentrations were manipulated prior to water immersion by appropriate dietary and exercise protocols (Martineau and Jacobs, 1989); the purpose of these studies was to evaluate the effects of very low and very high glycogen levels on metabolic heat production during the water immersion.

Metabolic rate during cold water immersion, expressed as oxygen consumption, increases to values that are usually around 4 or 5 times normal resting metabolic rate. Infrequently we have observed individuals who exhibit somewhat higher values, 6– or 7 times resting values. Our initial studies suggested that part of this increase in metabolic rate is fueled by muscle glycogen, as all of the subjects demonstrated a decrease in leg glycogen concentration after the water immersion (Martineau and Jacobs, 1988). The second objective of these experiments was to evaluate the effects of manipulating the pre-immersion glycogen levels on heat production during cold water immersion. Our manipulations did result in the subjects entering the water on one trial with muscle glycogen levels that were only about 50% of normal, and on another trial when they were about 150% of normal (Martineau and Jacobs, 1989). The oxygen consumption during the water immersion, was about the same on each trial. The respiratory exchange ratio (RER), however, differed between trials as expected.

Metabolic heat production is calculated based on the combination of RER and oxygen consumption. We observed significantly less metabolic heat production per unit time when the body's carbohydrate stores were depleted compared to the other trials (Martineau and Jacobs, 1989). There was also a significantly more rapid body cooling rate, as reflected by the changes in rectal temperature, when the body had little glycogen stored in its muscles, and presumably also in the liver.

These examples of some of our initial studies were done on subjects resting in cold air or cold water. In light of these findings we hypothesized that the requirement to do physical work superimposed on that cold stress might induce a more rapid breakdown of muscle glycogen than if the same work were done at a comfortable temperature. We therefore had subjects performing either light or heavy exercise once at 9°C air and again on a separate day at 21°C (Jacobs et al., 1985). We found that significantly more glycogen was in fact utilized to do the light exercise in the cold compared to doing the same work at 21°C. There was no difference in glycogen depletion rates, however, for the higher exercise intensities, and this is consistent with earlier observations that the heat production associated with hard exercise is sufficient to offset heat loss to the environment, thus obviating the need for shivering (Hong and Nadel, 1979).

We also carried out investigations of the effects of manipulating the body's circulating fat pools on heat production during cold water immersion. Vallerand and Jacobs (1990) reported that triglycerides infused intravenously were not eliminated more rapidly from the circulation during cold air exposure than during warm air exposure, contrasting with the results for glucose infusion (Vallerand et al., 1988). In another series of experiments, the circulating free fatty acid concentration was manipulated by having our subjects ingest nicotinic acid in the form of niacin pills prior to and during the water immersion (Martineau and Jacobs, 1989b). The effect of the nicotinic acid is to block lipolysis and this effect is demonstrated by the observation that the plasma free fatty acids and glycerol levels were dramatically reduced prior to, and during, the water immersion. Again contrasting with the effects of manipulating the carbohydrate stores, metabolic heat production was virtually unaffected; the proportion of the

total heat production that could be attributed to fat oxidation was significantly reduced, but there was compensation by simply increasing carbohydrate oxidation.

For reasons that are still unclear, carbohydrates seem to be a somewhat preferred substrate during shivering thermogenesis. There are similarities to hard physical exertion in that the body is not able to maintain the same intensity of exertion when carbohydrate stores are depleted, i.e. a shift to a greater reliance on fat oxidation to fuel muscle contraction is not sufficient for the musculature to be able to maintain a high level of exertion, just as body temperature could not be maintained as well when carbohydrate stores were depleted (Martineau and Jacobs, 1989a). We must mention that similar experiments were carried out at USARIEM and they did not detect any significant muscle glycogen utilization during cold water immersion (Young et al., 1989); we can not explain the discrepancies between our studies other than to suggest that perhaps the fact that our subjects were much leaner than those of Young et al. (1989) may be important in this regard.

Gender differences in response to cold stress have been the topic of a limited number of investigations and reviews (Stephenson and Kolka, 1993; Nunneley, 1978; Hayward et al., 1975) . It was reported that women cool faster than men during cold water immersion (Kollias et al., 1974; McArdle et al., 1984; Hessemer and Brück, 1985) , and this is somewhat surprising considering the greater body fat content of the average female. Body temperature changes associated with the menstrual cycle (Graham et al., 1989) , cardiovascular responses to rest and exercise (Stevens et al., 1987; Wagner and Horvath, 1985a,b) are other factors with associated gender differences in response to cold stress. To date potential gender-related physiological differences in responses to cold have not been considered in systematic studies such as those described above, i.e. quantification of the substrates used to fuel $\dot{M}$ during cold stress, nor in the development of $ST$ predictive models, including our own [Tikuisis, 1989; Tikuisis et al., 1988). Specifically, there are established gender differences in the ratio of lean body mass to total body mass and in the proportion of energy derived from carbohydrate or fat metabolism during exercise (Tarnopolsky et al., 1990).

There are, however, studies of gender differences with regard to skeletal muscle metabolism during exercise which suggest that untrained female musculature has an enzymatic profile which is predisposed to greater dependency on lipid metabolism than male muscle tissue (Green et al., 1984) . In male and female subjects matched for their physical training status, exertion at the same relative intensity is fueled by carbohydrate oxidation to a greater extent in males, and by lipid oxidation to a greater extent in females (Tarnopolsky et al., 1990; Phillips et al., 1993; Tarnopolsky et al., 1995) . Although potentially advantageous for endurance exercise, the evidence presented above relating to the importance of carbohydrate oxidation for shivering thermogenesis suggests that less carbohydrate oxidation may be disadvantageous in terms of *ST* in the cold. However, even if the magnitude of the increase in $\dot{M}$ may be less in females than males, the metabolic predisposition favoring lipid oxidation suggests that temperature regulation may not be as negatively influenced when glycogen availability is compromised. In terms of the muscle mass involved in shivering, models of human thermoregulation during cold stress use a fixed value to represent the contribution of the musculature of various body segments to the increase in $\dot{M}$ due to shivering. For example, this constant for the contribution of the trunk has previously only been estimated and ranged from 55-85% (Montgomery, 1974; Stolwijk, 1970; Hancock, 1980) . We recently experimentally determined this value for male subjects to be 71% (Bell et al., 1992), but here again no data are yet available for female subjects. The implications of these gender differences, if they apply to cold-induced increases in *M*, are potentially of sufficient magnitude to warrant their consideration in a model of *ST* in cold stressed females.

### 3.1 Objectives

This report is a progress report which describes the results from the first of a series of studies carried out to address the issues raised above. Specifically this study was designed to clarify the quantity and quality of energy substrate utilization in shivering female subjects during cold water immersion. The

objectives were: to determine the magnitude of $\dot{M}$ during cold water immersion; to quantify the relative contributions of fat, carbohydrate and protein metabolism to fueling $\dot{M}$; to determine if muscle glycogen is a significant energy source during shivering; to manipulate skeletal muscle glycogen availability and to determine the subsequent effects on $\dot{M}$ and body temperature regulation during cold stress.

### 3.2 Hypotheses

**a.** The relative increase in $\dot{M}$ caused by cold water immersion will be less in females than what has previously been reported for males.

**b.** Compared to previous reports for males, a smaller proportion of the increase in $\dot{M}$ will be due to increases in carbohydrate oxidation.

**c.** Contrasting with what has been reported for males, muscle glycogen depletion of large skeletal muscle groups will not impair body temperature regulation during cold water immersion.

## 4. METHODS

The protocol and methodology were chosen to enable comparison with data collected for male subjects using a similar protocol (Martineau and Jacobs, 1988,1989a). To facilitate these comparisons we restricted our metabolic studies to the use of indirect calorimetry, measurement of hormones and metabolites in venous blood, and measurement of metabolites in muscle biopsy samples.

Nineteen female subjects, aged 19-37, were recruited from local universities and within our research facility. Subjects did not donate blood for 30 days prior to or during participation in this study.

Subjects reported for their first visit having read a detailed information summary about all aspects of the study. They were given an opportunity to ask questions of the Scientific Authority and medical officers. Subjects then signed an informed consent and underwent a medical screening. Once receiving

clearance, physical characteristics including height and weight were determined and percent body fat was estimated after determination of body density by hydrostatic weighing. Maximum aerobic power ($\dot{V}O_2max$) was assessed separately for arm cranking exercise and for leg cycle ergometry so that appropriate exercise intensities for glycogen depletion exercise of both the upper and lower body could subsequently be determined. To familiarize the subjects with the laboratory setting and test procedures they were also immersed to the shoulders in 18°C water for 15 min.

### 4.1 Experimental design

On three subsequent visits the subjects were immersed in 18°C water, with identical procedures and measurements occurring for each cold exposure. The first immersion (control) was done after a 3-day period during which the subjects consumed an uncontrolled mixed diet. The two other experimental immersions followed 2.5 days of a specific dietary and/or exercise regimen (as described below) designed to elicit low or high glycogen levels in large skeletal muscle groups. The order of these dietary manipulations was counterbalanced among the subjects, with at least 6 days of uncontrolled mixed diet given between the two dietary regimes.

### 4.2 Standardization of menstrual and diurnal cycles

Initially, there was an attempt to have subjects undergo the various trials during the follicular phase of their menstrual cycle. However, the logistics of this timetable meant that some subjects would require two to three months to complete testing , which not only increased the likelihood of subject attrition but would also increase the risk of greater random trial-to-trial variation in measured responses. Moreover, Mittelman et al. (personal communication, 1997) recently showed that there was no effect of menstrual cycle phase on body temperature regulation during cold stress. We therefore felt that risk of significant random experimental error introduced by a long interval between immersions was greater than the risk that the menstrual cycle phases would confound our data interpretation. Thus, we did not

interpretation. Thus, we did not attempt to standardize the menstrual cycle phase for the various cold water immersions.

The subjects were always immersed at the same time of day to avoid possible diurnal effects. They were asked to abstain from alcohol for 48 hours before a trial, not exercise within 24 hours of a trial, and fast for 12-14 hours before each trial.

### 4.3 Glycogen depleting exercise

Three days before the two experimental immersions, two-legged exercise was performed for 60 min on a cycle ergometer at a power output requiring about 70% $\dot{V}O_2max$. This was followed by four or five 1-min exercise bouts performed at about 90% $\dot{V}O_2max$. This procedure has been demonstrated previously to result in depletion of glycogen from both main muscle fiber types of the leg muscles (Jacobs, 1981). After a 30-min rest period, the same exercise protocol was repeated using an arm-crank ergometer; the corresponding exercise intensities were about 60 and 90% arm $\dot{V}O_2max$.

### 4.4 Dietary manipulations

Immediately at the end of the exercise sessions, the subjects were instructed and informed about the composition of different types of food associated with carbohydrate (CHO)-reduced and CHO-rich diets. A list of suggested foods and a sample meal plan was provided to each subject. For 2.5 days the subjects consumed a free choice of foods within these guidelines. They were instructed verbally and with written guidelines that CHO should make up 90-95% of total nutritional consumption during the CHO-rich diet, but only 5-10% in the CHO-poor diet. The subjects were asked to weigh themselves daily, and to try to maintain the same total energy intake as usual throughout the diet treatment to ensure that body weight was being maintained. Previously, this laboratory used the same protocol to significantly alter the glycogen content in the vastus lateralis in males subjects (Martineau and Jacobs, 1989). Body weight was determined prior to each immersion.

### 4.5 Cold water immersion

On the day of each immersion the subjects reported to the lab in a 12-h post absorptive state, clad in a two-piece bathing suit. They inserted a rectal probe, were instrumented with 12 calibrated heat-flow transducers, bipolar ECG skin electrodes, and an intravenous catheter. They lay quietly in a supine position for 30 min at 23°C. Their resting metabolic rate was determined during the final 10 min of rest using a semi-automated metabolic cart system. Using an electrical winch system, they were lowered at a standardized rate into a water immersion calorimeter, with water temperature at 17.8°C, and remained in a supine position immersed to the neck in stirred chilled water until one of the following criteria, established before the experiment, was reached: 90 min elapsed, rectal temperature decreased to 35°C, or the subject asked to be withdrawn from the immersion tank.

### 4.6 Muscle biopsies

Muscle samples were taken from the right *quadriceps femoris vastus lateralis* just before water immersion (i.e. after the 30 minute rest period) and again from the same muscle just after each immersion, employing the percutaneous needle biopsy technique (Bergström, 1962). Skin and the underlying fascia were anaesthetized with 3 mL of xylocaine (2% epinephrine) after cleansing with an antiseptic solution (Betadine surgical scrub, Purdue Frederick Inc.). Both pre- and post immersion samples were taken from the same incision. Incisions were closed using Steri-Strip® (3M, St. Paul, MN). A water-proof dressing (Tegaderm®, 3M, St. Paul, MN) was placed over the Steri-Strip and then an elasticized bandage was wrapped around the thigh in an attempt to exert some pressure on the biopsy site and hopefully reduce the soreness that is frequently experienced in the thigh for 2-3 days after the biopsy. This bandage was left on the leg during the immersion, removed for the post-immersion biopsy and then dry Steri-Strips® and a dry elasticized bandage were placed on the leg after the experiment. Subjects were instructed to leave the elasticized bandage on the leg for 3-4 hours; they were instructed to leave the Steri-Strips® in place for 5 days.

During subsequent immersions, incisions were made on the same leg but at least 3 cm away from the previous incision.

On two occasions the Tegaderm® did not adhere well when the antiseptic residue around the biopsy site was not thoroughly removed with an alcohol swab prior to making the incision; when this was observed the subject's leg was quickly removed and elevated above the water line until a new bandage could be applied to cover the incision.

No complications, such as subsequent infection, resulted from the biopsies. Subjects did, however, report varying intensities of muscle soreness in the thigh, sometimes lasting as long as 4-5 days after the biopsy. The intensities ranged from no soreness at all to some subjects who were in extreme discomfort for 24 hours after the experiment. No subject requested or required follow-up medical referral. One subject did have a minor skin rash response to the Steri-Strip®. One subject reported loss of sensation in a 5 $cm^2$ skin area around the biopsy site, which is slowly resolving but is still evident almost one year after the experiment.

### 4.7 Blood sampling

The protocol called for venous blood samples to be obtained from an antecubital vein just before immersion (i.e. after the 30 min rest period) and after 5, 30, 60, and 90 min of immersion. Difficulties were encountered almost immediately in obtaining sufficient volume of blood, probably due to the combination of extreme vasoconstriction and decreased blood flow to the forearm. It is noteworthy that the incidence of problems in this regard was much higher than we have experienced when similar blood sampling methodologies were used with male subjects (Bourdon et al., 1995). Initially a 20 gauge, 1.0 inch catheter (Insyte™, Becton Dickinson) was used in conjunction with a slow infusion of warmed isotonic saline containing no heparin. Larger and longer catheters (20 gauge 8 inch and 18 gauge 1.25 inches) were also tried in an attempt to improve line patency, but without success. The best results were achieved using a heparin lock (10 U/mL) with the 20 gauge 1 inch catheter, and this system was used for the remainder of the experiment. A water-proof dressing

(Tegaderm®) was placed over the site where the catheter pierced the skin. During immersions the arm was supported just above the water by an adjustable sling hanging from the ceiling; this was done to reduce water contact with the catheter site and in an attempt to reduce local vasoconstriction. Ten mL blood samples were drawn and divided into 3 tubes which were kept on crushed ice: 2 mL were dispensed into heparin treated tubes for the subsequent determination of glucose, lactate, beta-hydroxy-butyrate, hematocrit and hemoglobin; 4 mL were expelled into a tube treated with EGTA (90 mg/mL) and glutathione (60 mg/mL), centrifuged and the plasma was frozen for subsequent determination of catecholamines; 4 mL were dispensed into a chilled, EDTA-treated tube (2 mL from this tube were in turn dispensed into a tube containing Trasylol® for subsequent determination of glucagon; the remaining 2 mL were centrifuged and aliquot of the plasma was subsequently used for the determination of free fatty acids, glycerol and insulin). All samples were stored at -20°C until frozen and then stored at -70°C until assayed.

### 4.8 Biochemistry

Hematocrit was determined by centrifugation (Autocrit Ultra3 centrifuge). Commercially available kits were used to measure concentrations of plasma glucagon (Glucagon RIA kit, Diagnostic Products Corporation, California), plasma insulin (Pharmacia Insulin RIA 100, Pharmacia, Uppsala, Sweden), and free fatty acids (WAKO™ NEFA kit, Texas). Glucose and hemoglobin were assayed using automated spectrophotometric techniques (Hemocue™). Plasma samples were analyzed for glycerol concentration after deproteinization (Boobis and Maughan, 1983), lactate and beta-hydroxy-butyrate (Maughan, 1982). Plasma epinephrine and norepinephrine levels were measured using negative ion chemical ionization gas chromatography-mass spectrometry (Zamecnik, 1997). Changes in plasma volume were calculated from the changes in hematocrit and hemoglobin concentration (Dill and Costill, 1974).

Muscle tissue samples were freeze dried for at least 8 hours. Glycogen was assayed as glucose units following hydrochloric acid hydrolysis using a fluorometric enzymatic method (Karlsson, 1971).

To facilitate calculations of protein oxidation during the immersions, the subjects were asked to collect urine for 24 h beginning the morning of, and prior to, the immersion. The urine was subsequently assayed for its urea nitrogen concentration (Sigma Kit 640, Sigma Chemicals Co., MO, USA).

**4.9 Temperature measurements**

The immersion tank used is a whole body calorimeter with exterior dimensions of 224.5 cm length by 93.2 cm width and 77.6 cm high. The volume of water in the calorimeter for the immersions was about 1200 liters. The calorimeter was initially developed to control water temperature and determine heat loss from an immersed object, however, in this experiment, water temperature was not controlled. Instead, subjects were immersed at a water temperature of 17.8°C and water temperature was measured continuously during the immersion, and for at least 30 minutes before and after each immersion. The temperature of the water was measured at 10 different sites using calibrated thermistors (unsheathed Baxter Rectal Probes, 400 series) and the water was stirred continuously. Heat loss from the body to the calorimeter was calculated by comparing the rate of water temperature change during the immersion to that measured before and after the immersion. For reference purposes, here are some data for one of the subjects to exemplify the resolution of the calculations of heat loss to the calorimeter: rate of water temperature prior to immersion was 0.092°C/h; during the first minute of immersion it increased to 1.458°C/h; 4 min into the immersion the value decreased to about 50% of that peak value, and by the last few minutes of immersion the change in water temperature was only 0.15°C/h. The water's heat gain due to the loss of heat from the webbed stretcher on which the subject lay was quantified and subtracted from all calculations. An automated data acquisition system was used to record the water temperatures at a frequency of 12 samples/min.

During the immersions the following were measured continuously with an automated data acquisition system, and averaged each minute: rectal temperature (Pharmaseal® 400 Series, Baxter Healthcare Corporation, California), mean skin temperature and mean skin heat flow using a 12-point area-weighted system as described elsewhere (Vallerand et al., 1989). For measurement of skin temperature and heat flow, the same twelve heat flow sensors (Concept Engineering, model FR-025-TH44033-F8-F, Connecticut) were used throughout the entire experiment.

### 4.10 Respiratory gas exchange measurements

Respiratory gases were monitored using a semi-automated metabolic cart system during the 30 min rest period prior to immersion, and continuously throughout the immersion, with the exception of a 5 minute break for re-calibration purposes after 25 min of immersion. For this purpose the subject was connected to a mouth-piece, breathing valve, and hose, which directed the expired gases to a 5 liter mixing box, which was connected in series to a ventilation module which measured expired ventilation rate (VMM Ventilation Measurement Module, Interface Associates, Irvine, California). A sample line directed gases from the mixing box to oxygen (AMETEK Model S-3A11, Applied Electrochemistry, Paoli, Pennsylvania) and carbon dioxide (AMETEK Model CD-3A, Applied Electrochemistry, Paoli, Pennsylvania) analyzers. Commercially available microcomputer based software (Vista/Turbofit Software, version 3.10, Vacumetrics Inc., Ventura, California) was used to register the data each minute, and to convert the values into STPD units of oxygen consumption and carbon dioxide production.

### 4.11 Calculation of metabolic heat production and substrate contributions

Metabolic heat production rates ($\dot{M}$) were calculated from the respiratory gas exchange measurements of oxygen consumption, carbon dioxide production, and the respiratory exchange ratio (RER) according to Pérronet et al. (1991) Data collected during the first 5 min of immersion were not used in any related

calculations because of the reflex hyperventilation caused by cold water immersion.

The rates of carbohydrate and fat oxidation ($CHO_{ox}$ and $FAT_{ox}$, respectively) were calculated using the non-protein oxygen consumption and the non-protein respiratory exchange ratio. Protein oxidation ($PRO_{ox}$) was assessed using the urinary urea nitrogen excretion rates (Vallerand et al., 1993). Detailed descriptions of the calculations for substrate oxidation rates are available in Vallerand et al. (1995).

**4.12 Statistical analyses**

Twelve subjects participated in the control immersion but only nine of them completed the other experimental immersions. The reasons for the subject attrition are described in the Results. In order to exploit as much data as possible the data were first analyzed to determine the effects of water immersion alone on the measured variables, without consideration of the dietary treatments. These data were analyzed using a one-factor analysis of variance for repeated measures. Of the twelve subjects completing the control immersions, only 10 completed 85 minutes of immersion and two subjects asked to be removed from the water after 60 min. Therefore, the control immersion data have been presented separately for two groups: a group (n=10) that completed 85 minutes of the control immersion (C85), and a group (n=12) that completed at least 60 min of immersion (C60).

The statistical analyses of the effects of the dietary treatments were then done using a data base of results from those subjects who completed all three experimental immersions (i.e. the control, high carbohydrate, and low carbohydrate immersions). These results were analyzed with a two-factor analysis of variance for repeated measures to determine the main effects of the immersion and the diet factors (Low, Control, or High), and any significant interactions between significant main effects. Of the nine subjects who completed all treatment immersions, one completed only 60 min of the control immersion. Therefore, these subjects were divided into two groups for the purpose of data

analysis and presentation. One group (n=9) completed at least 60 min of each trial (T60), and the other group (n=8) completed 85 min of every trial (T85). Statistical analysis was only performed on the groups that completed 85 min (C85 and T85). Unless otherwise noted, data are presented as mean values ± standard deviation. It was decided *a priori* that statistical significance would be accepted at the 95% confidence level.

## 5. RESULTS

### 5.1 Subject attrition

Nineteen subjects signed consent forms and completed all familiarization and medical screening procedures. Of these, seven subjects dropped out of the experiment: two due to pregnancy, two due to scheduling conflicts, and three because of the discomfort they perceived associated with the cold water immersion and/or the invasive procedures.

All of the remaining twelve subjects completed the control immersion and tolerated at least 60 min (C60); ten of these 12 completed the full 90 min of the control immersion (C85). The other two subjects voluntarily terminated the immersion at 60 min, one due to cold discomfort and the other due to the need to urinate and her unwillingness to do so in the immersion tank

Nine of the 12 subjects who did the control immersion also completed the other two experimental immersions. Three dropped out of the study at various stages, one because of apprehension about the biopsy procedure, one for medical reasons, and one due to scheduling conflicts.

Much of the data based on the respiratory gas exchange analysis (e.g. metabolic rate and substrate oxidation rates) are reported for an immersion duration of 85 min because the mouthpiece was removed from the subject at that time, thus the use of the terms C85 and T85 to denote subjects who lasted the full 90 min of immersion in the control and treatment trials, respectively.

The duration of each immersion for each subject is presented in Table 1.

## 5.2 Subject characteristics

The physical characteristics of the subjects are presented in **Table 2.** Subjects had a mean age of 24 y and were of average height and weight. The mean relative body fat mass was normal (23%), however, it ranged widely, from 14% in a competitive distance runner to 35%. With reference to $\dot{V}O_2max$, the subjects were of average fitness although two subjects had superior fitness levels due to their involvement in competitive sport. Only two subjects took oral contraceptives. As stated earlier the phase of the menstrual cycle on the day of each immersion was not standardized, but it was recorded and this information is presented in **Table 3.**

## 5.3 Rectal temperature and rate of body heat loss

*Control immersion.* **Figure 1** displays the change in rectal temperature for each subject during the control immersion, and **Figure 2** shows the mean response. Rectal temperature decreased and rate of body heat loss increased significantly during immersion. There was a wide range of response, with the change in rectal temperature being significantly correlated with the % body fat content (r=0.82). **Figure 3** depicts the calculated rate of heat loss to the calorimeter; as intuitively expected, maximal heat loss rates occurred during the first few minutes of immersion; by 30 minutes into the immersion vasoconstriction was probably close to maximal and the rates of heat loss were only about 25% of the initial rates and slowly decreased to even lower rates during the remaining portion of the immersion.

*Dietary treatment immersions.* There was a significant decrease in rectal temperature during all immersions but no difference among treatment trials (**Table 4**). **Figure 4** shows that there was a tendency for rectal temperature to reach a higher peak value during the high carbohydrate trial, and for rectal temperature to be somewhat lower during the latter phases of the immersion, but these trends were not statistically significant. Similarly there was no difference among trials for the rate of heat loss (**Figure 5**).

## 5.4 Metabolic heat production

*Control immersion.* Table 5 shows the values for $\dot{M}$ both before and during the immersion. Figure 6 shows the mean values for all subjects and demonstrates that $\dot{M}$ increased significantly about 2.5-fold during the first 10 min of immersion, and then continued to increase progressively but much more slowly, such that the final values were about 3.1 times the pre-immersion values. For the C85 group, the $\dot{M}$ data were grouped into 30 min intervals to facilitate statistical comparisons; the average $\dot{M}$ during the first 30 min of immersion was not different from the mean value for the data collected from 30-60 min of immersion. Both of these first two intervals were significantly lower than the $\dot{M}$ measured during the last 30 min of immersion (Figure 7). The peak metabolic rate recorded for each subject during the immersion was expressed relative to that subject's $\dot{V}O_2max$ during exhaustive exercise (Figure 8). There was no relationship between the $\dot{V}O_2max$ values and the % $\dot{V}O_2max$ elicited during shivering. During this immersion the percentages of $\dot{M}$ that were calculated to be derived from $CHO_{ox}$, $FAT_{ox}$ and $PRO_{ox}$, were 36±11%, 59±12%, and 5±3%, respectively. Both $CHO_{ox}$ and $FAT_{ox}$ increased significantly during the water immersion compared to the pre-immersion values, while there was no change in $PRO_{ox}$.

*Dietary treatment immersions.* $\dot{M}$ did not differ significantly when the various trials were compared (Table 6, Figure 9); the mean±SD values for $\dot{M}$ during the immersions were 123±30, 124±37, and 112±36 $W/m^2$ for the control, high carbohydrate, and low carbohydrate trials, respectively. $CHO_{ox}$ and $FAT_{ox}$, as a percent of the total $\dot{M}$, differed significantly across trials; % $\dot{M}$ due to $CHO_{ox}$ was significantly less both at rest and during immersion for the low carbohydrate trial than the other two trials, and % $\dot{M}$ due to $FAT_{ox}$ was significantly greater at rest and during immersion for the low carbohydrate trial than the other two trials (Figure 10). Individual values for the % $\dot{M}$ attributed to carbohydrate, fat and protein oxidation are shown in Table 7.

## 5.5 Muscle glycogen concentrations

*Control immersions.* Both pre and post immersion muscle biopsy samples were successfully done on 11 of the 12 subjects. The remaining subject did not have complete data because she complained of discomfort during the biopsy procedure; it was decided not to proceed with the biopsy rather than cause the subject more discomfort. Muscle glycogen levels were significantly lower after immersion in C85, the net difference amounting to a change of about 20% of the initial glycogen levels. **Table 8** shows that each of the subjects, with one exception, had a lower glycogen level after immersion when compared to the pre-immersion concentration. The subject who showed a net increase in glycogen was in the C60 group, i.e. she only remained in the water for 60 min during the control immersion. The mean magnitude of the change was similar in the C60 and C85 groups, which suggests that the rate of glycogen utilization may have been greater earlier in the immersion and that much slower glycogen utilization was occurring later in the immersion.

*Dietary treatment immersions.* In contrast with expectations, the exercise/nutritional treatments did not significantly change the pre-immersion muscle glycogen levels; they were similar on all trials. Mean glycogen concentrations (in mmol glucose units/kg dry muscle) prior to the Control, High and Low trials were 489±128, 573±147, and 460±74, respectively. The ANOVA resulted in a significant main effect of time, without any interaction with treatment. Thus, muscle glycogen decreased significantly as a result of immersion, but the dietary treatments did not affect the magnitude of that decrease (**Figure 11, Table 9**). The glycogen utilization rate was determined for group T85 by dividing the pre-post difference in glycogen concentration by 85 minutes, although it is acknowledged that the rate of change is probably not linearly related with elapsed time; this rate of change was also similar across trials with mean values of 1.09, 0.66 and 0.80 mmol glucose units/kg dry muscle/min during control, high carbohydrate and low carbohydrate trials, respectively.

## 5.6 Blood metabolites and hormones

As described in a previous section, difficulties were encountered in obtaining blood samples during immersion in several subjects. In light of the number of missing data at varying time intervals, statistical analysis was limited to comparing the pre with the post immersion values, and only for the five to six subjects for whom both samples were available.

*Control immersions.* With the exception of beta-hydroxy-butyrate and epinephrine, which did not change, there was a significant increase in all other metabolite and hormone concentrations. Glucose increased from 4.6±0.17 to 5.23±0.25 mmol/L. Insulin increased from 7.9±.96 to 10.7±1.3 μU/mL, while glucagon also increased slightly, but significantly, from 97±8 to 109±11 pg/mL. FFA and glycerol concentrations increased from 0.40±0.07 to 0.82±0.09 mmol/L, and from 0.057±0.007 to 0.17±0.020 mmol/L, respectively. Lactate increased from 0.9±0.2 to 2.1±0.4 mmol/L. The mean values for epinephrine were 39±19 before immersion and 121±73 ng/mL after immersion, a difference which just failed to reach statistical significance for the five subjects for whom both pre and post immersion samples were available ($p=0.08$). The corresponding values for norepinephrine were 229±30 and 1192±212 pg/mL, which was a significant difference.

These changes in blood metabolite and hormone concentrations should be considered in light of the hemoconcentration which was reflected in a 18% decrease in plasma volume, although some of the changes are too great to be attributed only to the hemoconcentration, e.g. glycerol (205%), insulin (35.4%), FFA (102%) and lactate (125%).

*Dietary treatment immersions.* The low carbohydrate treatment resulted in significantly higher pre-immersion concentrations for FFA, glycerol, and beta-hydroxy-butyrate, when compared to the pre-immersion values for the control and high carbohydrate trials. The exercise/dietary treatments did not significantly change the pre-immersion concentrations of glucose, lactate, insulin, glucagon, epinephrine or norepinephrine. The changes of the various metabolites and hormones were similar on all trials, with significant increases occurring in

concentrations of glucose, lactate, insulin, FFA, glycerol, beta-hydroxy-butyrate, epinephrine and norepinephrine but no significant change in glucagon. The magnitude of the increase in concentration should once again be considered in light of the significant decrease in plasma volume which occurred on all trials, to a similar degree: -15% during the high carbohydrate trial, -16 % during the low carbohydrate trial, and -20% during the control trial (**Table 10**).

The individual data for the various blood measurements are shown in **Tables 11-19**.

## 6. DISCUSSION

This document is a progress report describing the results of the first in a series of projects designed to investigate whether gender differences in physiological responses to cold stress are of a sufficient magnitude to have implications for predictive models of human body temperature regulation. The purpose of this particular project was to "...clarify the quantity and quality of energy substrate utilization in shivering female subjects during cold water immersion. The objectives were: to determine the magnitude of $\dot{M}$ during cold water immersion; to quantify the relative contributions of fat, carbohydrate and protein metabolism to fueling $\dot{M}$; to determine if muscle glycogen is a significant energy source during shivering; to manipulate skeletal muscle glycogen availability and to determine the subsequent effects on $\dot{M}$ and body temperature regulation during cold stress."

Detailed analysis, interpretation of the results, and the implications for predictive modelling will be reserved for the Final Report. Some commentary follows below, however, regarding the testing of the specific experimental hypotheses for this particular project.

6.1 Hypothesis A:

**"The relative increase in $\dot{M}$ caused by cold water immersion will be less in females than what has previously been reported for males."**

As noted in the results, metabolic rate increased during the control immersions to values that were about 3.2 times resting metabolic rate (RMR), corresponding to about 35% of the peak oxygen uptake during exhaustive exercise. Males who were exposed to the same water temperature achieved a metabolic rate that was only slightly higher, about 3.5 times their RMR, corresponding to about 30% of their peak oxygen uptake during exhaustive exercise (Martineau and Jacobs, 1988). Given the fact that the females in the present investigation had a relative body fat content that was at least double that of the males, and that the females were therefore relatively more insulated than the males used by Martineau and Jacobs (1988), the current study suggests that shivering induces relative increases in metabolic rate in females that are very similar to what has been reported for males. Thus this hypothesis is rejected.

When, however, the data are expressed relative to body surface area, the male subjects of Martineau and Jacobs (1988) generated heat from shivering at rates that were about 20% higher than the females. Perhaps, this is not too surprising given the likelihood that a unit of male body surface area covers proportionately more underlying skeletal muscle than is the case for females.

Skeletal muscle is the prime source of the increased heat production during shivering thus the absolute magnitude of heat production will of course be significantly higher in males than females. The implications for survival time in the cold may be more a function of the heat production per unit lean body mass than per unit of body surface area. Thus these data will be re-visited with more sophisticated statistical techniques in our final report, when attempts will be made to compare the heat production rates between genders on the basis of the relative activation of skeletal muscle used during shivering. Such comparisons will require the completion of our final study which employs electromyography.

6.2 Hypothesis B:

**"Compared to previous reports for males, a smaller proportion of the increase in $\dot{M}$ will be due to increases in carbohydrate oxidation."**

This hypothesis was based on reports about energy substrate utilization during exercise. Specifically, it has been documented that during exercise of similar relative intensities women oxidize more lipids, and therefore decrease carbohydrate and protein oxidation, compared with men (Tarnopolsky et al., 1990, 1995; Phillips et al., 1993). These results were observed in exercise intensities eliciting 65% and 85% of peak oxygen uptake.

Using a similar cold water immersion protocol with males the $\%\dot{M}$ attributed to carbohydrate oxidation was calculated to be 45-50% (Martineau and Jacobs, 1989a,b). In the current investigation with female subjects, it was calculated that the contribution of carbohydrate oxidation amounted to 38-40% of $\dot{M}$. It should be noted that muscle glycogen levels were significantly lower after immersion than before immersion for all trials, suggesting that muscle glycogen contributes to total carbohydrate oxidation during shivering in females, as was reported previously for male subjects.

The actual quantitative difference in heat production which would be associated with this difference in the relative contribution of carbohydrates to total energy expenditure must be extrapolated to longer duration cold water immersions in order to evaluate the implications for the prediction of survival times. This issue will be addressed in our Final Report. In the interim, this hypothesis is accepted.

6.3 Hypothesis C:

**"Contrasting with what has been reported for males, muscle glycogen depletion of large skeletal muscle groups will not impair body temperature regulation during cold water immersion."**

The results from the current investigation did not enable us to address this hypothesis because we were not successful in causing the subjects to deplete their muscle glycogen concentrations to levels comparable with what we previously

induced in male subjects. In spite of having attempted to use a protocol for glycogen depletion that was identical to that used by Martineau and Jacobs (1989a), muscle glycogen concentrations after the low carbohydrate dietary treatment were not significantly different than those on the other two trials. Interestingly, the high carbohydrate dietary treatment also did not result in the expected effect of increasing muscle glycogen levels. Since we depended on the subjects to follow our dietary guidelines, it is possible that their adherence was not satisfactory. However, this is not the first time that gender differences have been reported in attempts to manipulate muscle glycogen levels. Tarnopolsky et al. (1995) reported that in contrast to what was observed in male subjects who were exposed to an identical protocol, the females did not increase muscle glycogen levels with a protocol that has repeatedly been reported to cause quite dramatic increases in male subjects.

The dietary treatment in the current study did, however, have measurable effects on indices of energy metabolism both at rest and during the immersion. Specifically, the contribution of fat oxidation to total metabolic heat production was higher, and carbohydrate oxidation was lower, both at rest and during the immersion after the low carbohydrate dietary treatment when compared to the other two immersions. The absolute $\dot{M}$ was not affected, however, nor was the rate of change in rectal temperature different across trials.

## 7. SUMMARY AND CONCLUSIONS

A. Data collection for the first phase of the study was completed as scheduled. There were a couple of difficulties encountered which are noteworthy. The protocol used was a duplicate of one used previously with male subjects. It appeared to be much more arduous for the female subjects, resulting in significantly more subject attrition. Attrition was compensated by recruiting more subjects, so that we were successful in collecting data for the number of subjects originally projected. Vasoconstriction and lack of blood flow to the extremities resulted in more difficulty than expected in obtaining venous blood samples during the cold water immersion.

B. Shivering induced via cold water immersion results in relative increases in metabolic rate which are similar to those previously observed in male subjects. The absolute metabolic heat production for females will be significantly less than for the average male because of differences in body size; the implications in terms of body temperature regulation during more prolonged cold stress, will be analyzed with more sophisticated mathematical modelling techniques in our final report. Future analyses will consider the gender-related differences in body size and relative lean body mass.

C. Females used muscle glycogen as one of the carbohydrate energy stores to fuel shivering, as reported previously for male subjects. This investigation was not successful in addressing the issue of whether manipulations of glycogen levels prior to immersion would affect subsequent thermoregulation during cold stress. A protocol which has traditionally resulted in the ability to significantly manipulate muscle glycogen stores in male subjects, was ineffective when applied to the female subjects in this investigation. There is only one prospectively designed study which compared the responses of males and females to classical "glycogen loading" protocols, and it reported that females did not increase their muscle glycogen levels. Our investigation suggests that females may rapidly replete muscle glycogen stores after depletion, even when nutritional carbohydrate intake is very low.

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**Table 1.** Duration of immersion for all subjects during the control and immersion and during immersions after the low carbohydrate (CHO) and high CHO diets.

| Subject | Time (min) | | |
|---|---|---|---|
| | Control | High CHO | Low CHO |
| AA | 90 | 90 | 90 |
| TB | 90 | 90 | 90 |
| MD | 90 | 90 | 90 |
| HH | 90 | 90 | 90 |
| DL | 90 | 90 | 90 |
| GM | 60 | 90 | 90 |
| JN | 90 | 90 | 90 |
| MP | 90 | 90 | 90 |
| CS | 90 | 90 | 90 |
| AG | 90 | | |
| WH | 90 | | |
| KK | 60 | | |

Table 2. Physical characteristics of subjects completing all three trials (T60), 85 min of all three trials (T85), only the control trial (C60) and 85 min of the control trial (C85).

| Subject | HEIGHT (cm) | WEIGHT (kg) | AGE (yrs) | BSA ($m^2$) | BODY FAT (%) | Lean Mass (kg) | $VO_2$ max - arms (l/min) | $VO_2$ max - arms (ml/kg/min) | $VO_2$ max - arms (ml/kgLBM/min) | $VO_2$ max - legs (l/min) | $VO_2$ max - legs (ml/kg/min) | $VO_2$ max - legs (ml/kgLBM/min) | Oral Contraceptive |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AA | 178.8 | 70.2 | 20 | 1.874 | 21.4 | 55.18 | 1.64 | 22.8 | 29.72 | 2.71 | 37.9 | 49.11 | Yes |
| TB | 167.5 | 61.6 | 18 | 1.694 | 26.1 | 45.5 | 2.19 | 34.9 | 48.13 | 2.87 | 45.8 | 63.08 | No |
| MD | 160 | 59.4 | 30 | 1.616 | 27.1 | 43.3 | 1.08 | 16.6 | 23.28 | 1.73 | 28.3 | 39.95 | No |
| HH | 175.5 | 80.8 | 23 | 1.975 | 35 | 52.5 | 2.28 | 27.8 | 43.43 | 3.14 | 38.3 | 59.81 | No |
| DL | 170 | 61.7 | 37 | 1.709 | 14.3 | 52.88 | 1.93 | 30.6 | 36.50 | 3.13 | 49.9 | 59.19 | No |
| GM | 165.1 | 63.5 | 33 | 1.69 | 16.9 | 52.77 | 1.88 | 29.7 | 35.63 | 2.65 | 43 | 50.22 | No |
| JN | 151.5 | 63.3 | 19 | 1.588 | 32.3 | 42.85 | 1.3 | 20.3 | 30.34 | 2.04 | 32 | 47.61 | No |
| MP | 165 | 58.2 | 19 | 1.613 | 25.3 | 43.48 | 1.4 | 24.9 | 32.20 | 1.9 | 33.5 | 43.70 | No |
| CS | 169 | 65.3 | 22 | 1.719 | 19.6 | 52.5 | 1.8 | 26.6 | 34.29 | 2.6 | 39.2 | 49.52 | No |
| mean, T60 (n=9) | 166.93 | 64.89 | 24.56 | 1.72 | 24.22 | 49.00 | 1.72 | 26.02 | 34.83 | 2.53 | 38.66 | 51.35 | |
| ± SD | 8.07 | 6.91 | 6.98 | 0.13 | 6.85 | 5.06 | 0.40 | 5.59 | 7.43 | 0.52 | 6.85 | 7.77 | |
| mean, T85 (n=8) | 167.16 | 65.06 | 23.50 | 1.72 | 25.14 | 48.52 | 1.70 | 25.56 | 34.74 | 2.52 | 38.11 | 51.50 | |
| ± SD | 8.60 | 7.36 | 6.65 | 0.14 | 6.71 | 5.20 | 0.43 | 5.79 | 7.93 | 0.56 | 7.11 | 8.29 | |
| AG | 174 | 70.8 | 24 | 1.845 | 16.2 | 59.33 | 1.51 | 28.7 | 25.45 | 2.6 | 50.2 | 43.82 | Yes |
| WH | 167 | 70.9 | 23 | 1.792 | 21.6 | 55.59 | 1.58 | 22.3 | 28.42 | 1.8 | 25.4 | 32.38 | No |
| KK | 150.5 | 52.3 | 19 | 1.46 | 22.7 | 40.43 | 2.09 | 29.4 | 51.69 | 3.08 | 43.2 | 76.18 | No |
| mean, C60 (n=12 | 166.16 | 64.83 | 23.92 | 1.71 | 23.21 | 49.69 | 1.72 | 26.22 | 34.92 | 2.52 | 38.89 | 51.21 | |
| ± SD | 8.71 | 7.46 | 6.17 | 0.14 | 6.30 | 6.20 | 0.37 | 5.06 | 8.81 | 0.52 | 8.00 | 11.74 | |
| mean, C85 (n=10 | 167.83 | 66.22 | 23.50 | 1.74 | 23.89 | 50.31 | 1.67 | 25.55 | 33.18 | 2.45 | 38.05 | 48.82 | |
| ± SD | 9.13 | 7.81 | 5.74 | 0.15 | 6.27 | 6.42 | 0.38 | 5.18 | 9.24 | 0.55 | 8.28 | 12.31 | |

Table 3. Day of menstrual cycle during immersions.

| Subject | Day of Cycle During Immersion | | |
|---|---|---|---|
| | Control | High CHO | Low CHO |
| AA | 36 | 32 | 25 |
| TB | 3 | 20 | 8 |
| MD | 20 | 22 | 28 |
| HH | 23 | 26 | 5 |
| DL | 24 | 20 | 3 |
| GM | 7 | 19 | 12 |
| JN | 90+ | 90+ | 90+ |
| MP | | 6 | 2 |
| CS | 22 | 17 | 10 |
| AG | 2 | | |
| WH | 9 | | |
| KK | 21 | | |

**Table 4.** Mean rectal temperature during all three immersion trials.

| Time (min) | Tre ( °C) in group T60 | | | | | | Tre (°C) in group T85 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CONTROL | | HIGH CHO | | LOW CHO | | CONTROL | | HIGH CHO | | LOW CHO | |
| | Mean | ± SD | Mean | ± SD | Mean | ± SD | Mean | ± SD | Mean | ± SD | Mean | ± SD |
| Pre | 37.00 | 0.18 | 36.98 | 0.26 | 37.03 | 0.23 | 37.01 | 0.2 | 36.98 | 0.28 | 37.05 | 0.24 |
| 10 | 37.15 | 0.23 | 37.13 | 0.27 | 37.09 | 0.30 | 37.14 | 0.24 | 37.13 | 0.29 | 37.12 | 0.30 |
| 20 | 37.15 | 0.25 | 37.20 | 0.28 | 37.10 | 0.32 | 37.16 | 0.26 | 37.21 | 0.29 | 37.14 | 0.32 |
| 30 | 37.09 | 0.37 | 37.20 | 0.30 | 37.06 | 0.37 | 37.11 | 0.39 | 37.24 | 0.30 | 37.11 | 0.38 |
| 40 | 37.00 | 0.43 | 37.15 | 0.30 | 36.97 | 0.43 | 37.01 | 0.46 | 37.20 | 0.28 | 37.01 | 0.44 |
| 50 | 36.88 | 0.50 | 37.03 | 0.32 | 36.80 | 0.48 | 36.89 | 0.54 | 37.08 | 0.29 | 36.85 | 0.48 |
| 60 | 36.73 | 0.57 | 36.89 | 0.31 | 36.61 | 0.54 | 36.75 | 0.61 | 36.94 | 0.29 | 36.68 | 0.53 |
| 70 | | | | | | | 36.64 | 0.61 | 36.79 | 0.29 | 36.55 | 0.58 |
| 80 | | | | | | | 36.53 | 0.63 | 36.68 | 0.31 | 36.44 | 0.60 |
| 85 | | | | | | | 36.48 | 0.65 | 36.48 | 0.43 | 36.41 | 0.59 |

Table 5. Mean metabolic rate responses during the control immersion.

| Time (min) | $M$ ($W \cdot m^{-2}$) in C60 | | $M$ ($W \cdot m^{-2}$) in C85 | |
|---|---|---|---|---|
| | Mean | ± SD | Mean | ± SD |
| Pre | 43.24 | 4.22 | 43.48 | 4.45 |
| 10 | 108.34 | 22.22 | 106.52 | 23.90 |
| 20 | 120.36 | 42.06 | 107.12 | 31.41 |
| 30 | 129.09 | 38.90 | 118.52 | 31.62 |
| 40 | 129.82 | 37.07 | 120.22 | 31.29 |
| 50 | 129.72 | 34.17 | 123.87 | 33.42 |
| 60 | 131.67 | 27.51 | 128.60 | 27.97 |
| 70 | | | 138.45 | 25.17 |
| 80 | | | 137.32 | 31.82 |
| 90 | | | 140.10 | 26.52 |

Table 6. Mean metabolic rate responses during all three immersion trials.

| Time (min) | $M$ ($W \cdot m^{-2}$) in group T60 | | | | | | $M$. ($W \cdot m^{-2}$) in group T85 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CONTROL | | HIGH CHO | | LOW CHO | | CONTROL | | HIGH CHO | | LOW CHO | |
| | Mean | ± SD | Mean | ± SD | Mean | ± SD | Mean | ± SD | Mean | ± SD | Mean | ± SD |
| Pre | 43.67 | 4.34 | 43.09 | 6.07 | 41.17 | 2.71 | 44.20 | 4.31 | 42.51 | 6.22 | 40.79 | 2.63 |
| 10 | 108.92 | 24.01 | 119.76 | 38.99 | 94.42 | 35.47 | 108.70 | 25.65 | 113.62 | 36.74 | 92.78 | 37.55 |
| 20 | 114.03 | 39.75 | 127.62 | 49.04 | 110.21 | 38.87 | 105.63 | 32.86 | 117.74 | 41.76 | 105.36 | 38.53 |
| 30 | 127.70 | 42.09 | 131.42 | 53.82 | 116.23 | 40.44 | 118.22 | 33.14 | 118.91 | 41.24 | 110.64 | 39.33 |
| 40 | 128.34 | 40.06 | 135.83 | 60.18 | 113.30 | 42.15 | 119.69 | 32.62 | 120.63 | 41.99 | 107.24 | 40.65 |
| 50 | 126.84 | 37.12 | 128.42 | 56.17 | 110.79 | 38.14 | 120.43 | 33.94 | 113.55 | 36.48 | 103.95 | 34.37 |
| 60 | 129.53 | 30.37 | 134.34 | 52.07 | 122.55 | 35.90 | 124.98 | 29.00 | 121.04 | 35.75 | 116.60 | 33.30 |
| 70 | | | | | | | 134.79 | 23.93 | 130.96 | 37.78 | 123.77 | 35.72 |
| 80 | | | | | | | 130.54 | 27.50 | 133.23 | 35.62 | 121.58 | 33.84 |
| 85 | | | | | | | 139.25 | 28.12 | 141.80 | 29.98 | 124.49 | 31.18 |

Table 7. The relative proportion of metabolic heat production (%M) atttributed to carbohydrate, fat, and protein oxidation.

| | Carbohydrate oxidation (%M) | | | Fat oxidation (%M) | | | Protein oxidation (%M) | | |
|---|---|---|---|---|---|---|---|---|---|
| Subjects | Control | High | Low | Control | High | Low | Control | High | Low |
| GM | 51.60 | 76.78 | 60.61 | 44.67 | 20.90 | 33.42 | 3.72 | 2.33 | 6.06 |
| HH | 35.09 | 32.17 | 15.16 | 54.87 | 57.23 | 76.13 | 10.03 | 10.60 | 8.71 |
| MD | 30.88 | 41.89 | 27.79 | 66.82 | 55.37 | 68.05 | 2.30 | 2.74 | 4.16 |
| TB | 43.78 | 42.56 | 12.89 | 49.63 | 52.89 | 79.18 | 6.59 | 4.54 | 7.93 |
| MP | 36.42 | 31.77 | 22.15 | 58.72 | 66.38 | 73.08 | 4.86 | 1.86 | 4.77 |
| AA | 24.15 | 28.49 | 24.74 | 71.44 | 67.02 | 68.56 | 4.42 | 4.49 | 6.70 |
| DL | 58.17 | 32.45 | 29.93 | 38.39 | 64.69 | 66.04 | 3.44 | 2.85 | 4.03 |
| JN | 47.34 | 48.34 | 48.46 | 43.25 | 41.96 | 39.57 | 9.41 | 9.70 | 11.97 |
| CS | 26.03 | 36.01 | 11.29 | 68.15 | 59.65 | 82.40 | 5.82 | 4.34 | 6.31 |
| Mean, T60 | 39.27 | 41.16 | 28.11 | 55.10 | 54.01 | 65.16 | 5.62 | 4.83 | 6.74 |
| ±SD | 11.70 | 14.81 | 16.59 | 11.97 | 14.68 | 17.18 | 2.65 | 3.18 | 2.53 |
| Mean, T85 | 37.73 | 36.71 | 24.05 | 56.41 | 58.15 | 69.13 | 5.86 | 5.14 | 6.82 |
| ±SD | 11.49 | 6.84 | 12.04 | 12.09 | 8.37 | 13.24 | 2.73 | 3.25 | 2.69 |

Table 8. Muscle glycogen concentration (mmol glucose/kg dry muscle) pre and post control trial cold water immersion.
* indicates significant difference from pre ($p<0.05$)

| | Control | |
|---|---|---|
| Subject | Pre | Post |
| HH | 555.8 | 397.78 |
| AA | 500.38 | 400.58 |
| TB | 436.58 | 418.94 |
| MD | 285.06 | 274.27 |
| GM | 329.6 | 346.57 |
| CS | 705.33 | 510.1 |
| JN | 564.71 | 524.77 |
| MP | 474.41 | 435 |
| DL | 552.51 | 327.22 |
| AG | 414.85 | 342.8 |
| KK | 484.3 | 475.18 |
| Mean,(C60) | 482.14 | 404.84 |
| ± SD | 116.67 | 78.68 |
| Mean, (C85) | 498.85 | 403.5* |
| ± SD | 117.44 | 81.81 |

Table 9. Muscle glycogen concentration (mmol glucose•kg$^{-1}$ dry muscle) during three cold water immersion trials.
* indicates significant difference from pre for the specific immersion ($p<0.05$).

| | Control | | Low CHO | | High CHO | |
|---|---|---|---|---|---|---|
| Subjects | Pre | Post | Pre | Post | Pre | Post |
| HH | 555.8 | 397.78 | 322.58 | 257.84 | 521.26 | 464.76 |
| AA | 500.38 | 400.58 | 543.77 | 462.48 | 500.02 | 442.63 |
| TB | 436.58 | 418.94 | 500.72 | 464.8 | 903.09 | 756.67 |
| MD | 285.06 | 274.27 | 479.22 | 432.49 | 474.13 | 414.21 |
| GM | 329.6 | 346.57 | 440.85 | 262.79 | 580.61 | 500.77 |
| CS | 705.33 | 510.1 | 425.45 | 327.88 | 493.99 | 393.42 |
| JN | 564.71 | 524.77 | 569.88 | 415.48 | 718.12 | 674.51 |
| MP | 474.41 | 435 | 416.58 | 360.37 | 445.09 | 329.04 |
| DL | 552.51 | 327.22 | 440.1 | 403.25 | 412.95 | 519.15 |
| Mean, T60 | 489.38 | 403.91 | 459.91 | 376.38 | 561.03 | 499.46 |
| ± SD | 128.02 | 81.47 | 74.03 | 79.21 | 156.04 | 136.55 |
| Mean, T85 | 509.35 | 411.08* | 462.29 | 390.57* | 558.58 | 499.3* |
| ± SD | 120.95 | 84.01 | 78.77 | 71.39 | 166.63 | 145.98 |

**Table 10.** Change in blood plasma volume during cold water immersions.

| | Blood Plasma Volume (% change from rest) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Time (min) during Control Immersion | | | | Time (min) during High CHO Immersion | | | | Time (min) during Low CHO immersion | | | |
| SUBJECT | 5 | 30 | 60 | 90 | 5 | 30 | 60 | 90 | 5 | 30 | 60 | 90 |
| GM | | | | | | | | | -8.51 | -12.58 | -15.12 | -18.86 |
| TB | | | | | -5.65 | -6.28 | -13.1 | -11.37 | -5.16 | -12.71 | -9.59 | -18.91 |
| HH | -9.87 | -12.19 | -12.76 | -11.28 | -9.32 | -12.61 | -9.63 | -14.66 | -8.95 | -10.37 | -8.5 | -11.89 |
| MD | -15.66 | -12.14 | -16.49 | -21.99 | -8.77 | -11.64 | -11.66 | -17.53 | -6.66 | -7.26 | -6.66 | -18.29 |
| AA | -8.8 | -17.2 | -21.28 | -23.17 | | -13.26 | | -21.54 | -12.93 | -16.47 | -19.24 | -25.22 |
| JN | -11.62 | -14.99 | -21.26 | -36.13 | | | | | -9.01 | -12.46 | -17.75 | |
| DL | | -13.57 | -18.01 | -19.5 | | | -17.67 | -24.17 | -7.03 | -11.49 | -15.18 | -20.27 |
| MP | | | | | -1.79 | | -19.74 | | | | | |
| CS | -8.02 | -12.15 | | -20.42 | -6.97 | -8.66 | -11.17 | -19.5 | -1.3 | -10.76 | -12.36 | -15.94 |
| n | 5 | 6 | 5 | 6 | 5 | 5 | 6 | 6 | 8 | 8 | 8 | 7 |
| mean | -10.79 | -13.71 | -17.96 | -22.08 | -6.50 | -10.49 | -13.83 | -18.13 | -7.44 | -11.76 | -13.05 | -18.48 |
| ± SE | 0.61 | 0.34 | 0.71 | 1.34 | 0.60 | 0.59 | 0.67 | 0.77 | 0.42 | 0.33 | 0.57 | 0.58 |

**Table 11.** Changes in blood glucose levels before and after cold water immersions.

| | GLUCOSE (mmol/l) | | | | | |
|---|---|---|---|---|---|---|
| | CONTROL | | HIGH CHO | | LOW CHO | |
| Subject | PRE | POST | PRE | POST | PRE | POST |
| GM | 4.40 | • | 4.15 | • | 3.90 | 4.95 |
| TB | 4.85 | • | 4.55 | 5.40 | 4.45 | 5.15 |
| HH | 4.45 | 4.70 | 4.25 | 4.55 | 4.95 | 5.50 |
| MD | 3.85 | 4.60 | 4.25 | 4.55 | 4.25 | 4.70 |
| AA | 4.70 | 6.30 | 4.30 | 5.60 | 4.60 | 5.05 |
| JN | 4.85 | 5.20 | 4.90 | • | 4.80 | • |
| DL | 5.00 | 5.50 | 4.70 | 5.40 | 4.60 | 6.20 |
| MP | • | • | 4.60 | • | 4.80 | • |
| CS | 4.60 | 5.10 | 4.60 | 5.40 | 4.50 | 5.80 |

**Table 12.** Changes in blood lactate levels before and after cold water immersions.

| | LACTATE ($mM \cdot L^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | CONTROL | | HIGH CHO | | LOW CHO | |
| SUBJECT | PRE | POST | PRE | POST | PRE | POST |
| GM | 0.36 | • | 1.24 | • | 0.44 | 2.75 |
| TB | 0.56 | • | 0.99 | 1.11 | 0.68 | 1.65 |
| HH | 1.01 | 0.83 | 0.99 | 1.55 | 0.63 | 1.05 |
| MD | 0.58 | 1.77 | 0.86 | 0.80 | 0.74 | 1.67 |
| AA | 0.47 | 1.44 | 0.49 | 2.90 | 0.61 | 2.65 |
| JN | 1.70 | 1.91 | 1.08 | • | 1.09 | • |
| DL | 1.11 | 3.41 | 0.44 | 3.66 | 0.33 | 4.15 |
| MP | • | • | 0.42 | • | • | • |
| CS | 0.66 | 3.08 | 1.24 | 1.75 | 0.68 | 1.93 |

**Table 13.** Changes in blood insulin levels before and after cold water immersions.

| | INSULIN ($uU \cdot ml^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | CONTROL | | HIGH CHO | | LOW CHO | |
| SUBJECT | PRE | POST | PRE | POST | PRE | POST |
| GM | 3.50 | • | 5.41 | • | 4.20 | 7.18 |
| TB | 8.17 | • | 9.03 | 9.98 | 4.32 | 4.59 |
| HH | 7.65 | 9.28 | 7.65 | 8.91 | 7.87 | 8.06 |
| MD | 5.70 | 6.21 | 6.56 | 7.55 | 6.72 | 6.33 |
| AA | 5.21 | 8.77 | 5.26 | 9.08 | 5.92 | 9.54 |
| JN | 10.59 | 12.44 | 7.14 | • | 11.12 | • |
| DL | 7.44 | 14.12 | 3.00 | 8.84 | 3.93 | 10.97 |
| MP | • | • | 7.23 | • | • | • |
| CS | 10.70 | 13.30 | 6.23 | 11.07 | 5.13 | 8.43 |

Table 14. Changes in blood glucagon levels before and after cold water immersions.

| | GLUCAGON (pg•ml$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | CONTROL | | HIGH CHO | | LOW CHO | |
| | PRE | POST | PRE | POST | PRE | POST |
| GM | 74.03 | • | 82.99 | • | 80.76 | 97.38 |
| TB | 68.05 | • | 81.77 | 87.57 | 85.93 | 78.31 |
| HH | 98.18 | 114.67 | 90.15 | 96.79 | 96.41 | 97.76 |
| MD | 73.79 | 79.02 | 73.67 | 97.3 | 103.55 | 90.91 |
| AA | 96.24 | 98.69 | 90.08 | 106.94 | 100.44 | 104.45 |
| JN | 93.96 | 108.96 | 121.22 | • | 109.35 | • |
| DL | 120.81 | 145.54 | 101.79 | 146.98 | 116.44 | 146.05 |
| MP | • | • | 83.98 | • | • | • |
| CS | 122.18 | • | 100.38 | 115.95 | 108.06 | 128.42 |

Table 15. Changes in blood free fatty acid levels before and after cold water immersions.

| | FFA (mmol•l$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | CONTROL | | HIGH CHO | | LOW CHO | |
| Subject | PRE | POST | PRE | POST | PRE | POST |
| GM | 0.49 | • | 0.10 | • | 0.70 | 1.22 |
| TB | 0.45 | • | 0.41 | 0.76 | 0.76 | 1.17 |
| HH | 0.45 | 0.63 | 0.33 | 0.57 | 0.35 | 0.61 |
| MD | 0.55 | 1.06 | 0.35 | 0.83 | 0.57 | 0.94 |
| AA | 0.44 | 1.00 | 0.34 | 0.77 | 0.59 | 1.00 |
| JN | 0.36 | 0.65 | 0.29 | • | 0.41 | • |
| DL | 0.13 | 0.76 | 0.43 | 1.25 | 0.44 | 1.41 |
| MP | • | • | 0.66 | • | • | • |
| CS | 0.51 | • | 0.41 | 0.69 | 0.80 | 1.17 |

Table16 . Changes in blood glycerol levels before and after cold water immersions.

| | GLYCEROL (mmol•l$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | CONTROL | | HIGH CHO | | LOW CHO | |
| SUBJECT | PRE | POST | PRE | POST | PRE | POST |
| GM | 0.039 | • | 0.055 | • | 0.081 | 0.235 |
| TB | 0.044 | • | 0.040 | 0.121 | 0.045 | 0.150 |
| HH | 0.070 | 0.096 | 0.047 | 0.094 | 0.048 | 0.092 |
| MD | 0.051 | 0.194 | 0.046 | 0.119 | 0.058 | 0.167 |
| AA | 0.040 | 0.219 | 0.042 | 0.188 | 0.089 | 0.209 |
| JN | 0.085 | 0.135 | 0.076 | • | 0.094 | • |
| DL | 0.053 | 0.219 | 0.064 | 0.272 | 0.059 | 0.313 |
| MP | • | • | 0.074 | • | • | • |
| CS | 0.043 | 0.179 | 0.053 | 0.169 | 0.092 | 0.263 |

Table 17. Changes in beta-hydroxybutyrate levels before and after cold water immersions.

| | β-HB (mmol•l$^{-1}$)) | | | | | |
|---|---|---|---|---|---|---|
| | CONTROL | | HIGH CHO | | LOW CHO | |
| Subject | PRE | POST | PRE | POST | PRE | POST |
| GM | 0.17 | • | 0.08 | • | 0.44 | 0.70 |
| TB | 0.14 | • | 0.07 | 0.06 | 1.62 | 1.17 |
| HH | 0.10 | 0.11 | 0.09 | 0.09 | 0.10 | 0.18 |
| MD | 0.40 | 0.67 | 0.14 | 0.29 | 0.60 | 0.57 |
| AA | 0.22 | 0.45 | 0.13 | 0.41 | 0.48 | 0.73 |
| JN | 0.09 | 0.08 | 0.11 | • | 0.09 | • |
| DL | 0.08 | 0.11 | 0.31 | 0.60 | 0.24 | 0.50 |
| MP | • | • | 0.41 | • | • | • |
| CS | 0.28 | 0.40 | 0.09 | 0.13 | 0.56 | 0.77 |

Table 18. Epinephrine levels (ng•ml$^{-1}$) during three immersion trials.

| | Control | | | | | High CHO | | | | | Low CHO | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Subject | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 |
| AA | 40 | 118 | 113 | 46 | 189 | 28 | | 111 | | 147 | 59 | 204 | 193 | 139 | 132 |
| GM | 40 | | | | | 28 | | | | | 42 | 132 | 190 | 145 | 119 |
| TB | 217 | | | | | 87 | 67 | 113 | 115 | 29 | 67 | 67 | 92 | 27 | 41 |
| HH | 65 | 71 | 55 | 42 | 49 | 30 | 80 | 84 | 51 | 50 | 27 | 60 | 43 | 36 | 64 |
| MD | 47 | 11 | 95 | 98 | 169 | 16 | 34 | 64 | 51 | 19 | 24 | 66 | 56 | 51 | 49 |
| JN | 26 | 60 | | | 35 | 19 | | | | | 13 | | | | |
| DL | 17 | | 182 | 119 | 161 | 56 | | | 121 | 148 | 14 | 180 | 130 | | 225 |
| MP | | | | | | 24 | | | | | | | | | |
| CS | 18 | 72 | 91 | | | 19 | 82 | 59 | 48 | 34 | 21 | | 69 | 56 | 59 |

Table 19. Norepinephrine levels (pg•ml$^{-1}$) during three immersion trials.

| | Control | | | | | High CHO | | | | | Low CHO | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Subject | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 | 1 | 2 | 3 | 4 | 5 |
| AA | 194 | 642 | 974 | 758 | 1097 | 196 | | 1053 | | 900 | 151 | 670 | 732 | 989 | 1002 |
| GM | 369 | | | | | 747 | | | | | 441 | 1060 | 1858 | 2112 | 1923 |
| TB | 175 | | | | | 316 | 1272 | 2060 | 2704 | 2808 | 267 | 1524 | 2418 | 2954 | 2531 |
| HH | 209 | 678 | 599 | 814 | 925 | 265 | 622 | 556 | 832 | 1281 | 280 | 767 | 900 | 1015 | 872 |
| MD | 224 | 559 | 723 | 1007 | 1393 | 190 | 390 | 387 | 549 | 457 | 266 | 560 | 634 | 857 | 1642 |
| JN | 252 | 837 | | | 1122 | 192 | | | | | 233 | | | | |
| DL | 268 | | 1291 | 1355 | 1423 | 250 | | | 1398 | 1221 | 193 | | 958 | 1453 | 1337 |
| MP | | | | | | 330 | | | | | | | | | |
| CS | 228 | 799 | 963 | | | 248 | 755 | 906 | 1209 | 1345 | 263 | | 730 | 1304 | 1737 |

## 10. FIGURES

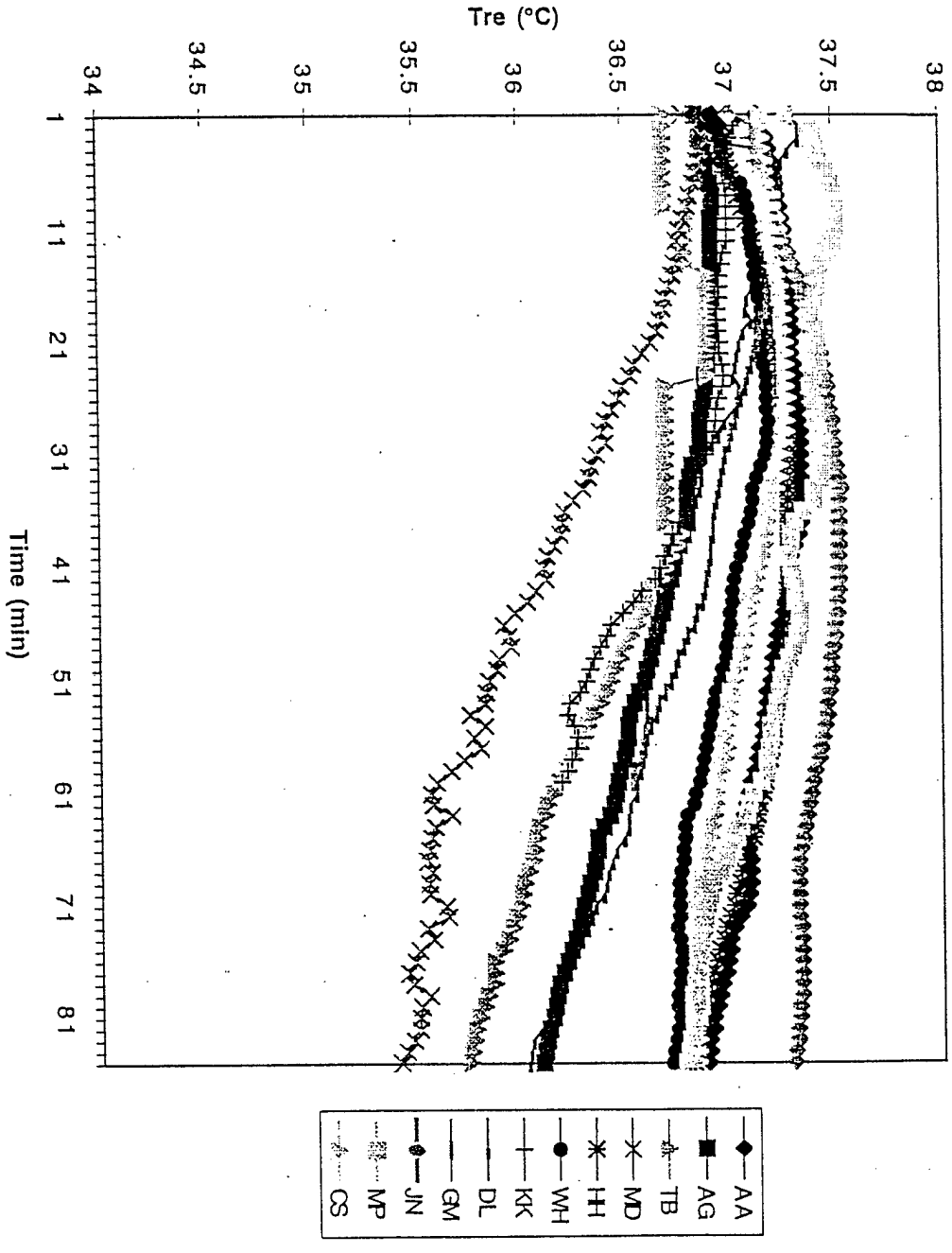



**Figure 1.** Individual rectal temperature response to cold water immersion.

**Figure 2.** Rectal temperature changes during control trial cold water immersion for subjects who endured 85 min (C85) or 60 min (C60) of immersion.

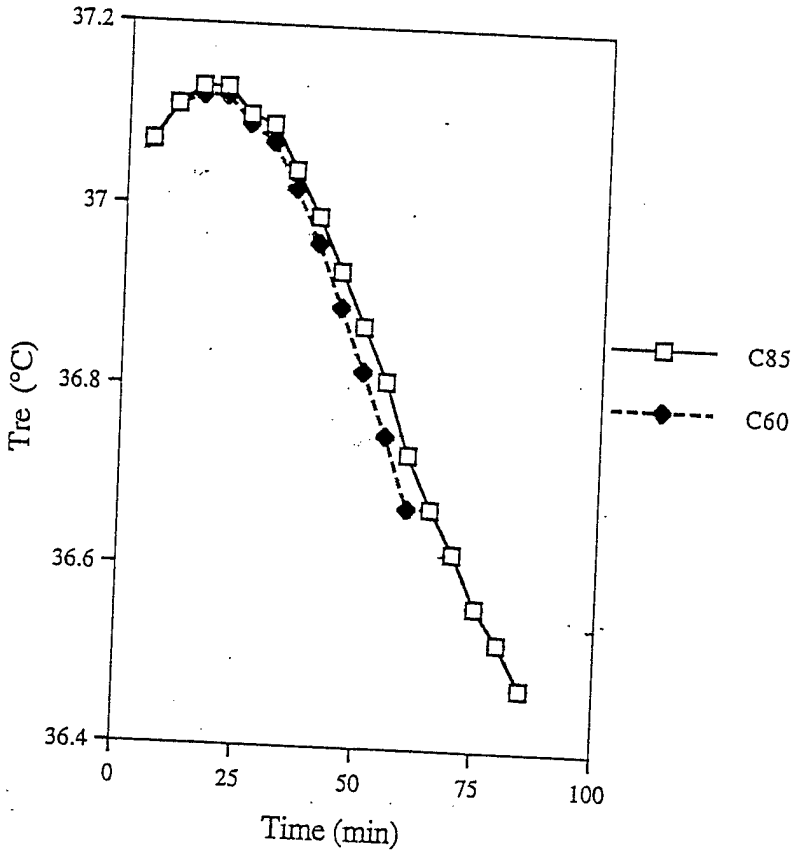


Figure 3. Mean heat storage during the control cold water immersion in C60 and C85.

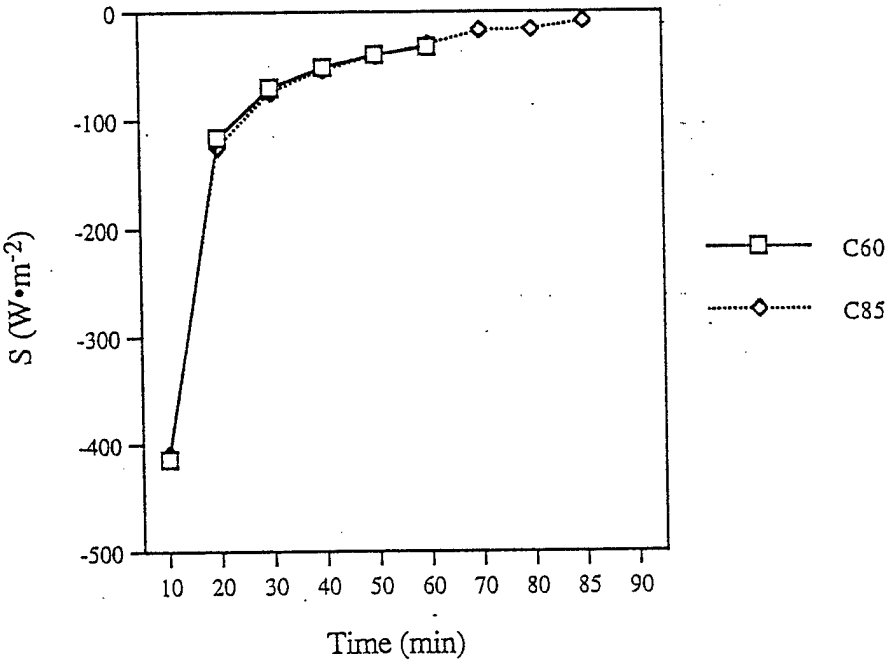


**Figure 4.** Mean rectal temperature changes during the high CHO, low CHO and control immersions in subjects completing 85 min (T85).

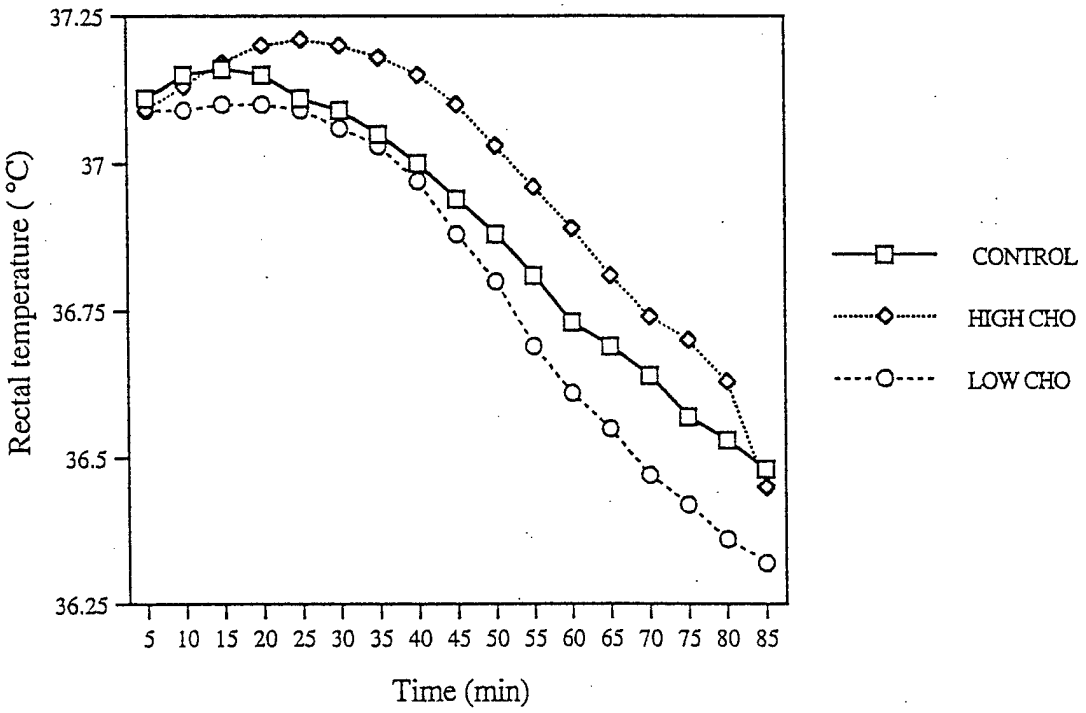


Figure 5. Mean heat storage changes during the three trials of cold water immersion in subjects completing 85 min (T85), n=8.

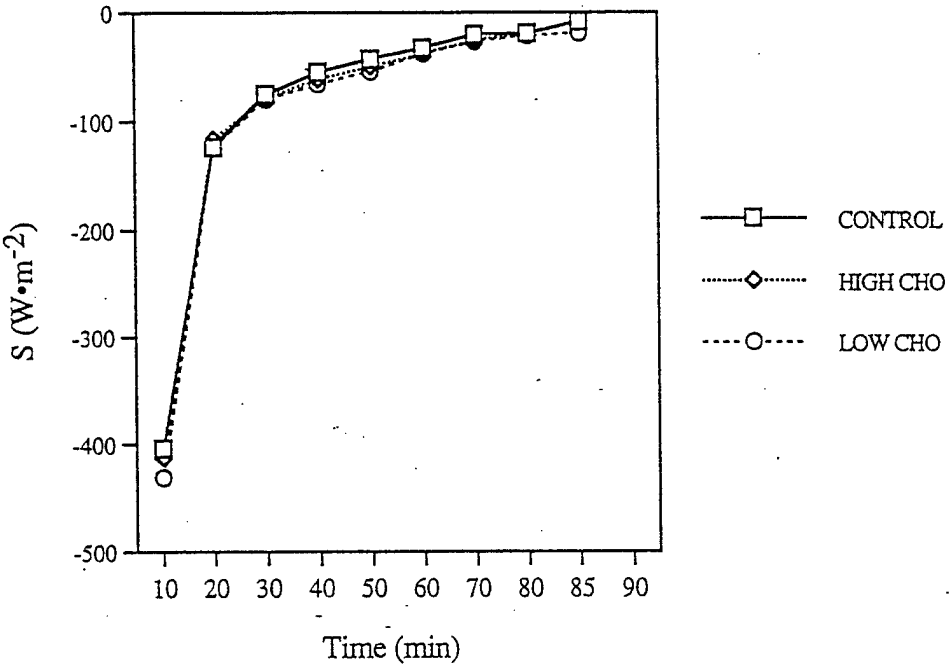


**Figure 6.** Metabolic rate response to cold water immersion in subjects who completed only 60 or 85 min of immersion.

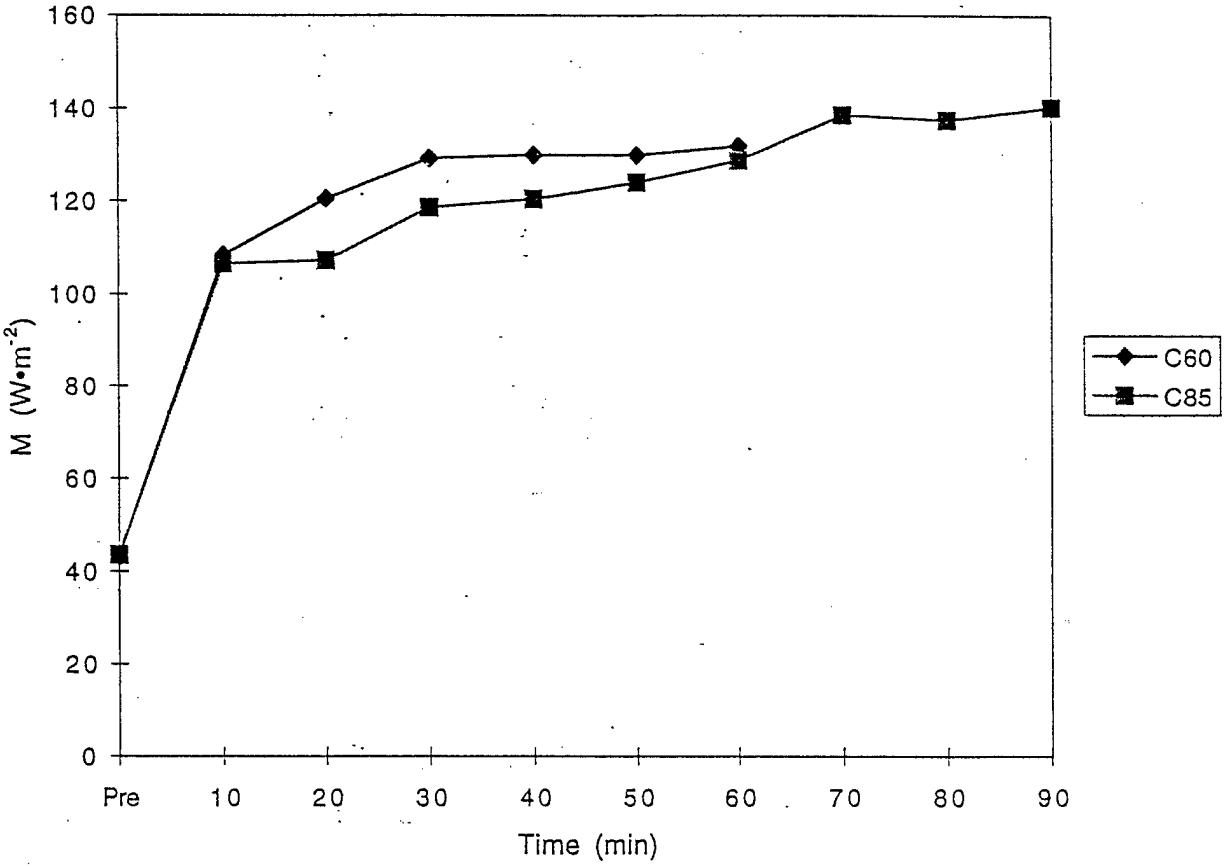


**Figure 7.** Metabolic rate response during cold water immersion.
*indicates significant difference from 60-90 ($p<.005$).

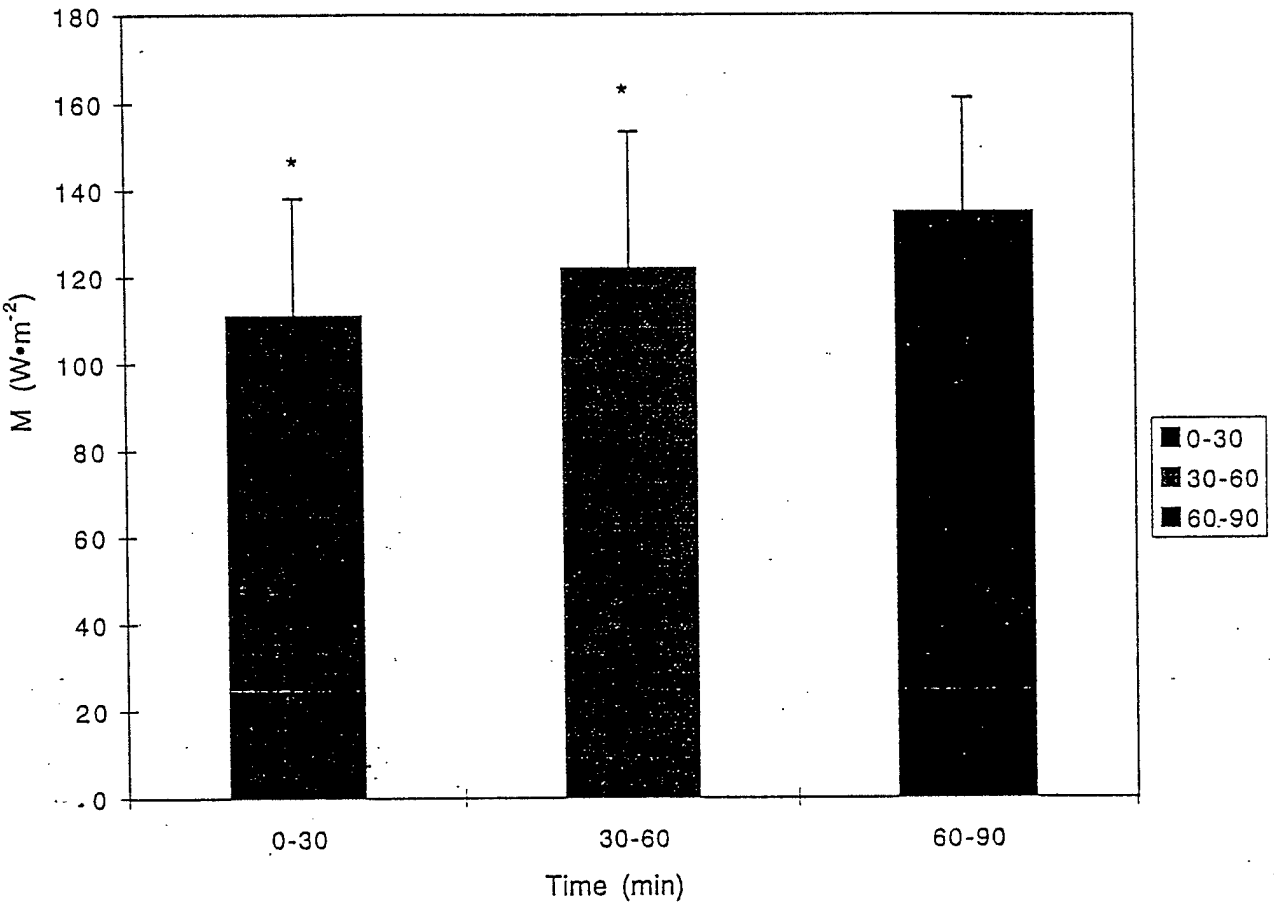


Figure 8. Individual subject data are shown for the peak rate of oxygen consumption during the control cold water immersion, expressed relative to maximal oxygen consumption rate during exhaustive exercise.

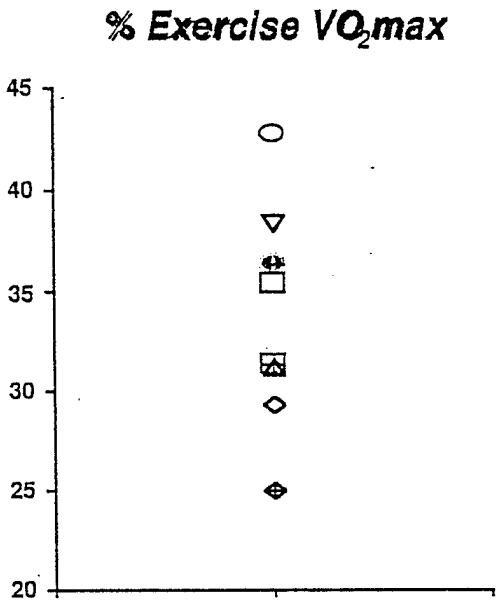


**Figure 9.** Comparison of metabolic rate during control, high CHO and low CHO immersions

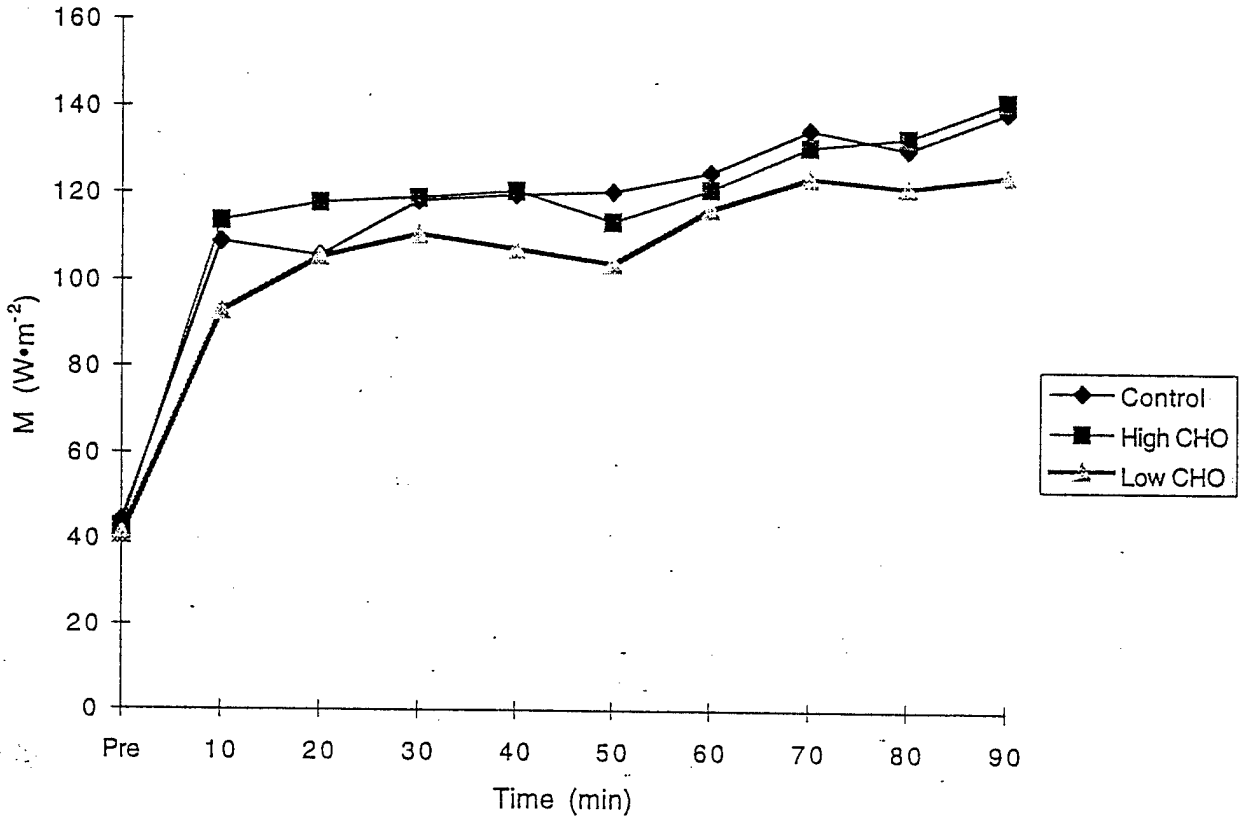
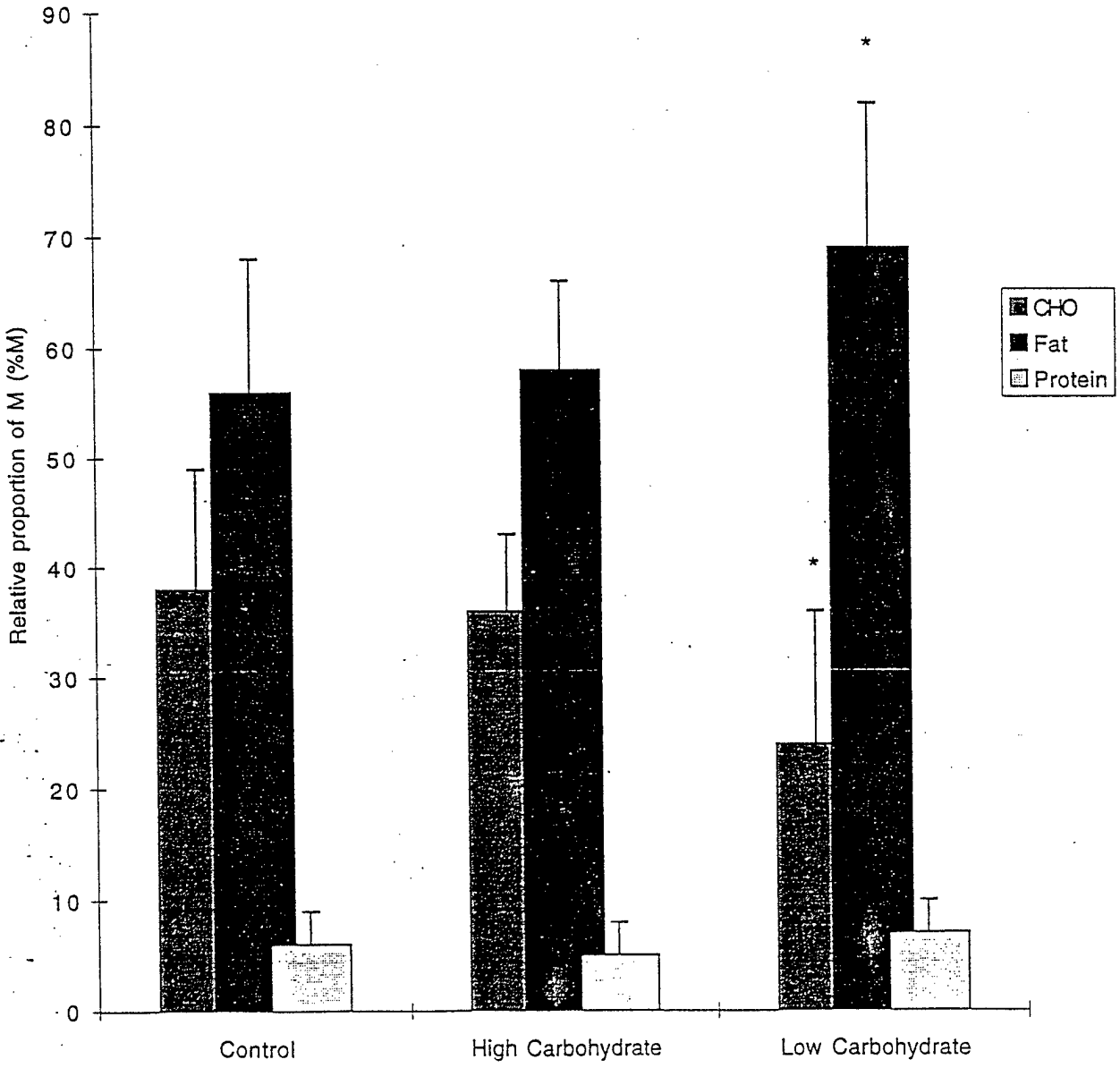


**Figure 10.** Substrate contribution to M (%M) by carbohydrate (CHO), fat and protein oxidation during control, high and low CHO immersions. *indicates that the low CHO trial was different from both the control and high CHO trials ($p<0.05$).



# ANNEX B

# ANNUAL REPORT 2

# 1. TABLE OF CONTENTS

## 2. ABSTRACT

This document is a progress report which describes the results from the second of a series of studies carried out to clarify the extent of gender-related differences in physiological responses to cold stress, and to evaluate the potential implications for survival time in the cold. Specifically, this study was designed to compare substrate utilization during light (LI) and moderate intensity (MI) exercise performed in both cold and comfortable ambient temperatures. The objectives were to quantify metabolic rate ($\dot{M}$), lipid and carbohydrate oxidation rates, and muscle glycogen utilization during light and moderate exercise in the cold versus comfortable ambient temperatures, and to compare these results with those previously collected in males who underwent the same protocol. Sixteen female subjects were divided into two groups matched for the submaximal exercise intensity corresponding to a blood lactate concentration of 4 mM (W4) during an incremental cycle exercise test. On two separate days subjects rested for 30 min at ambient temperatures of either 9 or 21°C, with the order of the trials balanced among subjects. Following rest a muscle biopsy was obtained from the m. vastus lateralis. Subjects in the MI group exercised for 30 min at 60%W4 while subjects in the LI group exercised for 30 min at 30%W4. Subjects exercised at the same power output for both trials. Another biopsy was taken immediately after exercise and both samples were assayed for glycogen concentration. $\dot{M}$ was significantly higher (13%) during exercise in the cold in LI but not MI. Muscle glycogen decreased significantly in MI (-28%) but not in LI; ambient temperature did not affect the magnitude of the change in muscle glycogen in either group. The relative contributions of carbohydrate, fat and protein oxidation to fueling $\dot{M}$ were similar in each exposure for both groups. These data provide further evidence of a more pronounced energy metabolism "predisposition" towards lipid metabolism in females than in males.

## 3. INTRODUCTION

Military units operate in cold air and cold water environments, and the associated training or missions can result in personnel being faced with life-threatening situations if they are ill-equipped or unprotected. As demonstrated by the recent winter crash in the Canadian Arctic of a military aircraft carrying infantry personnel, rescue can be delayed for days even when the precise location of survivors is known (de Groot, 1994). Cold water immersion hypothermia recently caused the deaths during training of US Army Rangers (Fort Benning, 1995). In light of such potential emergencies the prediction of survival time (*ST*) in the cold, defined in this document as the elapsed time until the onset of lethal hypothermia, is essential to meet the needs of Search and Rescue authorities. Such predictions are also useful in the analysis of strategic human factors demands of military operations in the cold, to prepare for contingencies of such operations, and to evaluate the potential benefits of equipment/clothing designed to protect the soldier from the cold.

An understanding of *ST* in healthy, sedentary, non-traumatized individuals is based in the following relationships. Once the protective insulation of available shelter or clothing is maximized, cold-stressed humans elevate metabolic heat production ($\dot{M}$) by shivering in an attempt to balance heat loss. Existing models of *ST* in cold air or cold water are based on observations of factors which affect $\dot{M}$ and the rate of heat loss from the body. In such models $\dot{M}$ increases as a function of temperature signals from the core and skin. When cold exposure is too severe for $\dot{M}$ to balance heat loss, *ST* is largely determined by the rate of heat loss from the body. Where there is a balance between $\dot{M}$ and heat loss, *ST* is limited by the endurance time for shivering.

The physiological factors characterizing $\dot{M}$ are relatively complex. Until about a decade ago there was very little empirically based information available in this regard for human subjects. Research has demonstrated that the relationship

between *ST*, $\dot{M}$, and heat loss is affected by the extent of the muscle mass involuntarily recruited during shivering (Bell et al., 1992), connective heat transfer during cold stress (Tikuisis et al., 1991), muscle substrate availability (Jacobs et al., 1994), the type and quantity of substrate oxidized by shivering musculature (Vallerand and Jacobs, 1989), and body composition (Tikuisis et al., 1988). Our research during the last decade has focused on such factors with the objective of generating sufficient knowledge to improve the predictive modeling of *ST* in the cold. A brief review of this research follows.

By measuring the electrical activity of many muscle groups simultaneously during cold-induced shivering, we demonstrated that several large muscle groups are recruited and contract at relatively low intensities that are less than 20% of their maximum force generating capabilities (Bell et al., 1992). Since so many muscle groups are involved in shivering, the sum total of their contractile activities can result in a four or five-fold increase in metabolic rate, and heat production.

Much of our attention has been directed towards the substrates that are used by skeletal muscle to increase heat production during shivering. For example, Vallerand et al. (1988) administered a clinical glucose tolerance test to subjects who were sitting in either cold air or at a comfortable temperature for two hours. These data were the first to show in humans that glucose is eliminated more rapidly from the circulation during cold exposure, presumably to provide more available substrate to fuel the increase in metabolic rate. It is also noteworthy that this more rapid uptake of glucose during cold exposure occurs with lower insulin levels in the cold compared to warm temperatures.

We subsequently continued to attempt to quantify the rates of substrate oxidation of fat, carbohydrate and protein in humans during cold exposure with indirect calorimetric techniques. As one might presume, the increase in metabolic rate during shivering is caused by increases in oxidation of both fat and carbohydrate, but the relative increase in the rate of substrate oxidation caused by shivering is greatest for carbohydrates (Vallerand and Jacobs, 1989). In resting subjects exposed to either cold air or cold water, carbohydrates and fat contribute

approximately equally to heat production (Martineau and Jacobs, 1991; Vallerand and Jacobs, 1989). From a strategic point of view, this finding seems unfortunate because the body's availability of carbohydrates is quite limited compared to the abundant fat and protein stores. We were already aware of the well established positive relationship between muscle glycogen concentration and endurance exercise performance of skeletal muscle and speculated that there may be a similar detrimental effect caused by muscle glycogen depletion on another form of muscle contraction, i.e. shivering and the associated heat production.

We therefore carried out a series of studies on male subjects immersed in 18°C water. The subjects were removed from the water when their rectal temperature reached 35.5° C. Biopsies were taken from the thigh muscle before and after the immersion to evaluate the changes in glycogen as a result of the water immersion (Martineau and Jacobs, 1988). In another study muscle glycogen concentrations were manipulated prior to water immersion by appropriate dietary and exercise protocols (Martineau and Jacobs, 1989); the purpose of these studies was to evaluate the effects of very low and very high glycogen levels on metabolic heat production during the water immersion.

Metabolic rate during cold water immersion, expressed as oxygen consumption, increases to values that are usually around 4 or 5 times normal resting metabolic rate. Infrequently we have observed individuals who exhibit somewhat higher values, 6– or 7 times resting values. Our initial studies suggested that part of this increase in metabolic rate is fueled by muscle glycogen, as all of the subjects demonstrated a decrease in leg glycogen concentration after the water immersion (Martineau and Jacobs, 1988). The second objective of these experiments was to evaluate the effects of manipulating the pre-immersion glycogen levels on heat production during cold water immersion. Our manipulations did result in the subjects entering the water on one trial with muscle glycogen levels that were only about 50% of normal, and on another trial when they were about 150% of normal (Martineau and Jacobs, 1989). The oxygen consumption during the water immersion, was about the same on each trial. The respiratory exchange ratio (RER), however, differed between trials as expected.

Metabolic heat production is calculated based on the combination of RER and oxygen consumption. We observed significantly less metabolic heat production per unit time when the body's carbohydrate stores were depleted compared to the other trials (Martineau and Jacobs, 1989). There was also a significantly more rapid body cooling rate, as reflected by the changes in rectal temperature, when the body had little glycogen stored in its muscles, and presumably also in the liver.

These examples of some of our initial studies were done on subjects resting in cold air or cold water. In light of these findings we hypothesized that the requirement to do physical work superimposed on that cold stress might induce a more rapid breakdown of muscle glycogen than if the same work were done at a comfortable temperature. We therefore had subjects performing either light or heavy exercise once at 9°C air and again on a separate day at 21°C (Jacobs et al., 1985). We found that significantly more glycogen was in fact utilized to do the light exercise in the cold compared to doing the same work at 21°C. There was no difference in glycogen depletion rates, however, for the higher exercise intensities, and this is consistent with earlier observations that the heat production associated with hard exercise is sufficient to offset heat loss to the environment, thus obviating the need for shivering (Hong and Nadel, 1979).

We also carried out investigations of the effects of manipulating the body's circulating fat pools on heat production during cold water immersion. Vallerand and Jacobs (1990) reported that triglycerides infused intravenously were not eliminated more rapidly from the circulation during cold air exposure than during warm air exposure, contrasting with the results for glucose infusion (Vallerand et al., 1988). In another series of experiments, the circulating free fatty acid concentration was manipulated by having our subjects ingest nicotinic acid in the form of niacin pills prior to and during the water immersion (Martineau and Jacobs, 1989b). The effect of the nicotinic acid is to block lipolysis and this effect is demonstrated by the observation that the plasma free fatty acids and glycerol levels were dramatically reduced prior to, and during, the water immersion. Again contrasting with the effects of manipulating the carbohydrate stores, metabolic heat production was virtually unaffected; the proportion of the

total heat production that could be attributed to fat oxidation was significantly reduced, but there was compensation by simply increasing carbohydrate oxidation.

For reasons that are still unclear, carbohydrates seem to be a somewhat preferred substrate during shivering thermogenesis. There are similarities to hard physical exertion in that the body is not able to maintain the same intensity of exertion when carbohydrate stores are depleted, i.e. a shift to a greater reliance on fat oxidation to fuel muscle contraction is not sufficient for the musculature to be able to maintain a high level of exertion, just as body temperature could not be maintained as well when carbohydrate stores were depleted (Martineau and Jacobs, 1989a). We must mention that similar experiments were carried out at USARIEM and they did not detect any significant muscle glycogen utilization during cold water immersion (Young et al., 1989); we can not explain the discrepancies between our studies other than to suggest that perhaps the fact that our subjects were much leaner than those of Young et al. (1989) may be important in this regard.

Gender differences in response to cold stress have been the topic of a limited number of investigations and reviews (Stephenson and Kolka, 1993; Nunneley, 1978; Hayward et al., 1975) . It was reported that women cool faster than men during cold water immersion (Kollias et al., 1974; McArdle et al., 1984; Hessemer and Brück, 1985) , and this is somewhat surprising considering the greater body fat content of the average female. Body temperature changes associated with the menstrual cycle (Graham et al., 1989) , cardiovascular responses to rest and exercise (Stevens et al., 1987; Wagner and Horvath, 1985a,b) are other factors with associated gender differences in response to cold stress. To date potential gender-related physiological differences in responses to cold have not been considered in systematic studies such as those described above, i.e. quantification of the substrates used to fuel $\dot{M}$ during cold stress, nor in the development of $ST$ predictive models, including our own [Tikuisis, 1989; Tikuisis et al., 1988). Specifically, there are established gender differences in the ratio of lean body mass to total body mass and in the proportion of energy derived from carbohydrate or fat metabolism during exercise (Tarnopolsky et al., 1990).

There are, however, studies of gender differences with regard to skeletal muscle metabolism during exercise which suggest that untrained female musculature has an enzymatic profile which is predisposed to greater dependency on lipid metabolism than male muscle tissue (Green et al., 1984) . In male and female subjects matched for their physical training status, exertion at the same relative intensity is fueled by carbohydrate oxidation to a greater extent in males, and by lipid oxidation to a greater extent in females (Tarnopolsky et al., 1990; Phillips et al., 1993; Tarnopolsky et al., 1995) . Although potentially advantageous for endurance exercise, the evidence presented above relating to the importance of carbohydrate oxidation for shivering thermogenesis suggests that less carbohydrate oxidation may be disadvantageous in terms of *ST* in the cold. However, even if the magnitude of the increase in $\dot{M}$ may be less in females than males, the metabolic predisposition favoring lipid oxidation suggests that temperature regulation may not be as negatively influenced when glycogen availability is compromised. In terms of the muscle mass involved in shivering, models of human thermoregulation during cold stress use a fixed value to represent the contribution of the musculature of various body segments to the increase in $\dot{M}$ due to shivering. For example, this constant for the contribution of the trunk has previously only been estimated and ranged from 55-85% (Montgomery, 1974; Stolwijk, 1970; Hancock, 1980) . We recently experimentally determined this value for male subjects to be 71% (Bell et al., 1992), but here again no data are yet available for female subjects. The implications of these gender differences, if they apply to cold-induced increases in $\dot{M}$, are potentially of sufficient magnitude to warrant their consideration in a model of *ST* in cold stressed females.

### 3.1 Objectives

This report is a progress report which describes the results from the second of a series of studies carried out to address the issues raised above. Specifically, this study was designed to compare substrate utilization during light and moderate exercise performed in both cold and comfortable ambient temperatures.

The objectives were: to quantify $\dot{M}$, lipid and carbohydrate oxidation rates, and muscle glycogen utilization during light and moderate exercise in the cold; to compare the results to when the same exercise is performed a comfortable temperatures; to compare all results with those previously collected in males who underwent the same protocol.

### 3.2 Hypotheses

**a.** $\dot{M}$ will increase to a greater extent when light exercise is performed during cold air exposure than when performing the same exercise at a comfortable temperature.

**b.** Contrasting with what has been reported for males, this increase will not be associated with any greater muscle glycogen utilization than is the case when the same exercise is performed at a comfortable temperature.

## 4. METHODS

The protocol and methodology were chosen to enable comparison with data collected for male subjects using a similar protocol (Jacobs et al. 1985). To facilitate these comparisons we restricted our metabolic studies to the use of indirect calorimetry, measurement of hormones and metabolites in venous blood, and measurement of metabolites in muscle biopsy samples.

Sixteen female subjects, aged 19-37, were recruited from local universities and within our research facility. Subjects did not donate blood for 30 days prior to or during participation in this study.

Subjects reported for their first visit having read a detailed information summary about all aspects of the study. They were given an opportunity to ask questions of the Scientific Authority and medical officers. Subjects then signed an informed consent and underwent a medical screening. Once receiving medical clearance, physical characteristics including height and weight were determined and percent body fat was estimated after determination of body

density by hydrostatic weighing. Subjects performed an exercise test to near exhaustion on an electrically braked cycle ergometer to determine the power output at a lactate concentration of 4 mmol/l (W4). W4 is an index of aerobic fitness and exercise intensity (Jacobs et al. 1995, Jacobs, 1986) and was used in this study to determine the exercise intensity at which subjects in each group would perform. During this exercise test, subjects began cycling at 30 or 60 Watts and intensity was increased by 30 watts every 4 min. Blood was drawn from the ear lobe during the last 30 s of each 4 min interval. W4 was interpolated from a plot of lactate concentration against power output. Metabolic measurements were made throughout the test.

### 4.1 Experimental design

The subjects were divided into two groups, equally matched for the W4 scores. The low intensity (LI) group exercised at 30% W4 and the moderate intensity (MI) group exercised at 60% W4. On two subsequent visits the subjects exercised for 30 min following a 30 min supine rest, once at a room temperature of 9°C and once at 21°C, with identical procedures and measurements occurring for each exposure. The order of the exposures was counter-balanced among subjects with at least 7 days between trials.

### 4.2 Standardization of menstrual and diurnal cycles

There was no attempt to control for menstrual cycle phase. Mittelman et al. (personal communication, 1997) recently showed that there was no effect of menstrual cycle phase on body temperature regulation during cold stress. We therefore felt that risk of significant random experimental error introduced by a long interval between exposures was greater than the risk that the menstrual cycle phases would confound our data interpretation.

The subjects were tested at the same time of day to avoid possible diurnal effects. They were asked to abstain from alcohol for 48 hours before a trial, not exercise within 24 hours of a trial, and fast for 12-14 hours before each trial.

### 4.3 Exercise and Exposure

On the day of each exposure the subjects reported to the lab in a 12-h post absorptive state, clad in shorts, t-shirt socks and running shoes. They inserted a rectal probe, were instrumented with 12 calibrated heat-flow transducers, bipolar ECG skin electrodes, and an intravenous catheter. They lay quietly in a supine position for 30 min at either 9°C or 21°C. Their resting metabolic rate was recorded for 10 minutes beginning after 15 minutes of rest in a supine position using a semi-automated metabolic cart system. Rectal temperature, heat flow and skin temperatures were also measured during this time. The first biopsy was taken after these measurements were made, and after the muscle the subjects mounted the cycle ergometer and cycled for 30 min at an intensity of either 30% or 60% of W4 depending on the group to which they were assigned. Metabolic rate, heat flow and skin temperature were measured continuously during the 30 min of exercise. Heart rate and ratings of perceived exertion were recorded every 5 min.

### 4.4 Muscle biopsies

Muscle samples were taken from the right *quadriceps femoris vastus lateralis* just before exposure (i.e. after the 30 minute rest period) and again from the same muscle within 45 seconds after completing exercise, employing the percutaneous needle biopsy technique (Bergström, 1962). Skin and the underlying fascia were anaesthetized with 3 mL of xylocaine (2% epinephrine) after cleansing with an antiseptic solution (Betadine surgical scrub, Purdue Frederick Inc.). Both pre- and post exposure samples were taken from the same incision. Incisions were closed using Steri-Strip® (3M, St. Paul, MN). An elasticized bandage was wrapped around the thigh in an attempt to exert some pressure on the biopsy site and hopefully reduce the soreness that is frequently experienced in the thigh for 2-3 days after the biopsy. This bandage was left on the leg during the exposure, removed for the post-exposure biopsy and then dry Steri-Strips® and a dry elasticized bandage were placed on the leg after the experiment. Subjects were instructed to leave the elasticized bandage on the leg for 3-4 hours; they were

instructed to leave the Steri-Strips® in place for 5 days. During the subsequent exposure, incisions were made on the same leg but at least 3 cm away from the previous incision.

No complications, such as subsequent infection, resulted from the biopsies. Subjects did, however, report varying intensities of muscle soreness in the thigh, sometimes lasting as long as 4-5 days after the biopsy. The intensities ranged from no soreness at all to some subjects who were in extreme discomfort for 24 hours after the experiment. No subject requested or required follow-up medical referral. One subject experienced some residual bleeding into her clothing because the Steri-Strips® did not adhere to the leg when she left the lab.

**4.5 Blood sampling**

The protocol called for venous blood samples to be obtained from an antecubital vein just before and after the 30 min rest and at 15 and 25 min during exercise. Difficulties in obtaining sufficient volume of blood were sometimes encountered during the cold exposure, probably due to the combination of vasoconstriction and decreased blood flow to the forearm. A heparin lock (10 U/mL) with the 20 gauge 1 inch catheter was used. A water-proof dressing (Tegaderm®) was placed over the site where the catheter pierced the skin to help stabilize the catheter. Ten mL blood samples were drawn and divided into 4 tubes which were kept on crushed ice: 5 mL were expelled into a tube treated with EGTA (90 mg/mL) and glutathione (60 mg/mL), centrifuged and the plasma was frozen for subsequent determination of catecholamines; 5 mL were dispensed into a chilled, EDTA-treated tube (50 µL were dispensed into tubes containing $HClO_4$ for the subsequent determination of glucose and lactate; samples were taken to determine hematocrit and hemoglobin; the remainder was centrifuged and aliquots of the plasma was subsequently used for the determination of free fatty acids and glycerol). All samples were stored at -20°C until frozen and then stored at -70°C until assayed.

### 4.6 Biochemistry

Hematocrit was determined by centrifugation (Autocrit Ultra3 centrifuge). Commercially available kits were used to measure concentrations of free fatty acids (WAKO™ NEFA kit, Texas). Glucose and hemoglobin were assayed using automated spectrophotometric techniques (Hemocue™). After deproteinization samples were analyzed for glycerol (Boobis and Maughan, 1983) and lactate (Maughan, 1982). Plasma epinephrine and norepinephrine levels were measured using negative ion chemical ionization gas chromatography-mass spectrometry (Zamecnik, 1997). Changes in plasma volume were calculated from the changes in hematocrit and hemoglobin concentration (Dill and Costill, 1974).

Muscle tissue samples were freeze dried for at least 8 hours. Glycogen was assayed as glucose units following hydrochloric acid hydrolysis using a fluorometric enzymatic method (Karlsson, 1971).

To facilitate calculations of protein oxidation during exposure, the subjects were asked to collect urine for 24 h beginning the morning of, and prior to, the rest and exercise in the environmental chamber. The urine was subsequently assayed for its urea nitrogen concentration (Sigma Kit 640, Sigma Chemicals Co., MO, USA).

### 4.7 Temperature measurements

During 15 to 25 min of the rest period and continuously throughout the exercise, the following were measured with an automated data acquisition system and averaged each minute: rectal temperature (Pharmaseal® 400 Series, Baxter Healthcare Corporation, California), mean skin temperature and mean skin heat flow using a 12-point area-weighted system as described elsewhere (Vallerand et al., 1989). For measurement of skin temperature and heat flow, the same twelve, calibrated heat flow sensors (Concept Engineering, model FR-025-TH44033-F8-F. Connecticut) were used throughout the entire experiment.

### 4.8 Respiratory gas exchange measurements

Respiratory gases were monitored using a semi-automated metabolic cart system between 15 and 25 min of the 30 min rest period and continuously throughout the exercise. For this purpose the subject was connected to a mouth-piece, breathing valve, and hose, which directed the expired gases to a 5 litre mixing box, which was connected in series to a ventilation module which measured expired ventilation rate (VMM Ventilation Measurement Module, Interface Associates, Irvine, California). A sample line directed gases from the mixing box to oxygen (AMETEK Model S-3A11, Applied Electrochemistry, Paoli, Pennsylvania) and carbon dioxide (AMETEK Model CD-3A, Applied Electrochemistry, Paoli, Pennsylvania) analyzers. Custom designed computer software (DCIEM/HPP Metabolic Measurement System V1.0, Keefe and Pope, 1997) was used to register the data each minute, and to convert the values into STPD units of oxygen consumption and carbon dioxide production.

### 4.9 Calculation of metabolic heat production and substrate contributions

Metabolic heat production rates ($\dot{M}$) were calculated from the respiratory gas exchange measurements of oxygen consumption, carbon dioxide production, and the respiratory exchange ratio (RER) according to Pérronet et al. (1991).

The rates of carbohydrate and fat oxidation ($CHO_{ox}$ and $FAT_{ox}$, respectively) were calculated using the non-protein oxygen consumption and the non-protein respiratory exchange ratio. Protein oxidation ($PRO_{ox}$) was assessed using the urinary urea nitrogen excretion rates (Vallerand et al., 1993). Detailed descriptions of the calculations for substrate oxidation rates are available in Vallerand et al. (1995).

### 4.10 Statistical analyses

The reasons for the subject attrition are described in the Results. Comparisons between the LI and MI groups were made using a one-factor analysis of variance while intragroup comparisons were made using a repeated measures

analysis of variance. Unless otherwise noted, data are presented as mean values ± standard deviation. It was decided *a priori* that statistical significance would be accepted at the 95% confidence level.

## 5. RESULTS

### 5.1 Subject attrition

Eighteen subjects signed consent forms and completed all familiarization and medical screening procedures. Of these, two subjects dropped out of the experiment due to scheduling conflicts.

### 5.2 Subject characteristics

The physical characteristics of the subjects are presented in **Table 1**. Subjects had a mean age of 25 y and were of average height and weight. The mean relative body fat mass was normal (23%) and subjects were of average fitness (W4=121Watts). Subject characteristics were not significantly different between groups. Only three subjects took oral contraceptives, two in the LI group and one in the MI group. As stated earlier the phase of the menstrual cycle on the day of each immersion was not standardized, but it was recorded and this information is presented in **Table 2**.

### 5.3 Temperature measurements and heat flow

There was no significant difference in initial rectal temperature (Tre) taken upon arrival at the laboratory between groups for each exposure (**Table 2**), despite the fact that we did not control for menstrual cycle phase. **Table 3** shows the rectal temperature (Tre) responses for each subject during each exposure and **Figure 1** illustrates Tre during each exposure for each group for each minute of exercise. Ambient temperature during the rest period did not significantly affect Tre. Furthermore, during exercise, there was no effect of ambient temperature on Tre within each group at any time. The mean value for Tre in LI was higher at rest at 9°C than at 21°C but this was not statistically significant; one subject showed

an unusually high increase in Tre during the rest period at 9°C which was associated with an unusually high average metabolic rate.

To determine the effect of exercise intensity and ambient temperature on Tre, delta Tre was calculated as a change in mean Tre (averaged over each 10 min interval of exercise) from the Tre during the first minute of exercise. These values are illustrated in **Figure 2.** These data show that the increase in Tre during exercise was lowest in LI (0.123°C and 0.22°C during the 9°C and 21°C trials, respectively) and greatest in MI (0.31°C and 0.7°C during the 9°C and 21°C exposures, respectively. Delta Tre increased ($p<0.03$) between each time interval during each exposure except in LI at 9°C when there was no significant increase in Tre between 10 and 20 min. Delta Tre in LI was greater at 21°C($p<0.05$) compared to 9°C at 20 and 30 min but not at 10 min. In MI, delta Tre was greater at 21°C than 9°C at all times. **Table 4** shows mean heat storage for each group during each exposure. Heat storage was lower during rest at 9°C compared to 21°C ($p<0.0001$). There was a significant effect of both ambient temperature and exercise intensity on heat storage ($p<0.02$). Heat storage was lowest in LI at 9°C (28.9±17.6 $W \cdot m^{-2}$) followed by LI at 21°C (58.1±15.6 $W \cdot m^{-2}$). The highest heat storage occurred in MI (78.9±28.4 and 124.9±17.6 $W \cdot m^{-2}$ at 9°C and 21°C, respectively). The mean heat flow for each group during each exposure is shown in **Table 5.** Heat flow was significantly lower during rest and exercise at 9°C compared to 21°C, however there was no effect of exercise intensity. Mean skin temperature for each group during each exposure is provided in **Table 6.** As expected, skin temperature was lower during rest at 9°C (28±.7 °C) than at 21°C (31±.7 °C). There was no effect of exercise intensity on skin temperature. When the groups were considered as one, skin temperature decreased with time at 9°C and increased with time at 21°C ($p<0.0001$).

### 5.4 Metabolic heat production and substrate contributions

The rate of metabolic heat production ($\dot{M}$) for each subject during each exposure is provided in **Table 7.** **Figure 3** illustrates the mean $\dot{M}$ for each group

during each of the exposures. During the rest period, $\dot{M}$ was significantly higher ($p<0.005$) at 9°C (94±25 $J \cdot s^{-1}$ or 57±13 $W \cdot m^{-2}$) than at 21°C (77±15 $J \cdot s^{-1}$ or 46±7 $W \cdot m^{-2}$). During exercise, $\dot{M}$ was greater at 9°C compared to 21°C ($p<0.02$) in LI but not MI. LI elicited a mean $\dot{M}$ of 308±55 $J \cdot s^{-1}$ or 184±25 $W \cdot m^{-2}$ at 21°C whereas at 9°C the same exercise intensity elicited a mean $\dot{M}$ of 348±52 $J \cdot s^{-1}$ or 209±30 $W \cdot m^{-2}$. There was no significant difference for the MI group between $\dot{M}$ at 21°C (424±65 $J \cdot s^{-1}$ or 261±37 $W \cdot m^{-2}$) and 9°C (432±54 $J \cdot s^{-1}$ or 265±30 $W \cdot m^{-2}$).

Substrate oxidation for each subject during each exposure is provided in **Table 8**. Neither exercise intensity nor ambient temperature significantly affected the type of substrate used during each exposure. When substrates were grouped together, substrate oxidation rates were greater ($p<0.005$) at 9°C (31±25 $J \cdot s^{-1}$) than 21°C ( 26±16 $J \cdot s^{-1}$) during the rest period prior to exercise. During exercise, there was an effect of ambient temperature on substrate oxidation in LI but not MI ($p<0.02$). Substrate utilization was greater in LI at 9°C (116±92 $J \cdot s^{-1}$) than at 21°C (103±78 $J \cdot s^{-1}$. **Figure 4** illustrates mean substrate oxidation as a percentage of $\dot{M}$ in order to demonstrate the relative contributions of each of the substrates to fueling metabolism during each of the trials. There was no difference in the relative contributions of each of the substrates to fueling $\dot{M}$ in either group during exercise at either 9°C or 21°C. Averaged over both trials, $CHO_{ox}$ contributed 62±11%, $Fat_{ox}$ contributed 36±11% and $PRO_{ox}$ contributed 2.4±0.8% to fueling $\dot{M}$.

### 5.5 Muscle glycogen concentrations

Muscle glycogen content before and after each of the trials is provided for each subject in **Table 9** and mean values are displayed in **Figure 5**. We were unable to obtain a post-exercise muscle sample in one subject in LI therefore her data were excluded from statistical analysis and data is presented as mean±SE. There was no effect of ambient temperature on the magnitude of change in glycogen in either group. When the data from both the 9°C and the 21°C trials were pooled together, mean muscle glycogen content decreased by 26% in MI, from 390±29 (mean±SE) to 289±21 mmol glucose·kg dry $muscle^{-1}$ ($p<0.03$). The change in glycogen levels in LI

was not significant, 434 ± 18 (mean±SE) before exercise and 405 ± 19 mmol glucose·kg dry muscle$^{-1}$ after exercise.

### 5.6 Blood metabolites and hormones

Blood metabolite and hormone levels for each subject during each exposure are shown in **Tables 10 to 16**. As described previously, some difficulties were encountered in withdrawing blood samples in the cold air and therefore there are some missing data. Only those subjects for whom all samples were available were included in the statistical analysis. Hemoglobin (Hgb) increased significantly ($p<0.01$) during rest by 6% at 9°C and 3% at 21°C. During exercise, there was a significant effect of exercise intensity ($p<0.01$) but not ambient temperature. Hgb increased by 4% in LI and 7.7% in MI. Hematocrit (Hct) increased ($p<0.01$) during rest at 9°C (8.3%) but not 21°C. There was no effect of either ambient temperature or group on Hct during exercise however, when exposures and groups were averaged together, Hct increased ($p<0.0001$) during exercise by 10%. Lactate increased ($p<0.05$) during rest at 9°C (22%) but not 21°C. There was no effect of group on lactate concentrations. When data during exercise was pooled from the two groups, lactate increased ($p<0.05$) at both 21°C (104%) and 9°C (136%). At the end of exercise, lactate was greater ($p<0.05$) at 9°C compared to 21°C. There was no effect of either group or ambient temperature on NEFA or glycerol during rest or exercise. When groups and exposures were averaged together, NEFA increased ($p<0.0001$) by 25% during rest and by 48% during exercise. Glycerol increased ($p<0.0001$) by 38% during rest and 104% during exercise. There was no effect of ambient temperature on glucose, however, the MI group had pre-rest glucose levels that were significantly lower than the LI group and only the MI group showed a decrease during rest. When the data were pooled from both groups, there was a small, but significant decrease ($p<0.02$) in glucose.

These changes in blood metabolite and hormone concentrations should be considered in light of the hemoconcentration which is outlined for each subject

during each exposure in **Table 10.** Changes in lactate, NEFA and glycerol were too large to be attributed only to hemoconcentration.

Catecholamine levels have not yet been analyzed but will be included in the Final Report.

## 6. DISCUSSION

This document is a progress report describing the results of the second in a series of projects designed to investigate whether gender differences in physiological responses to cold stress are of a sufficient magnitude to have implications for predictive models of human body temperature regulation. The objectives were: to quantify $\dot{M}$, lipid and carbohydrate oxidation rates, and muscle glycogen changes during light and moderate exercise in the cold; to compare the results to when the same exercise is performed at comfortable temperatures; to compare all results with those previously collected in males who underwent the same protocol.

Detailed analysis, interpretation of the results, and the implications for predictive modeling will be reserved for the Final Report. Some commentary follows below, however, regarding the testing of the specific experimental hypotheses for this particular project.

### 6.1 Hypothesis A:

**"$\dot{M}$ will increase to a greater extent when light exercise is performed during cold air exposure than when performing the same exercise at a comfortable temperature."**

Shivering is the prime source of thermogenesis in humans during cold exposure. As such, it was expected that light exercise performed in the cold, at a metabolic rate which is typically lower than that observed during shivering, would not be sufficient to offset the requirement for additional shivering thermogenesis. As outlined in the results, $\dot{M}$ was indeed greater during exercise at 9°C compared to 21°C in LI but not in MI. Compared to the 21°C exposure, $\dot{M}$

was 13% higher at 9°C in LI and only 1.7% higher in MI. This would indicate that shivering was present when subjects performed only light intensity exercise at 9°C and that heat production during moderate intensity exercise was sufficient to preclude the need for shivering (Hong and Nadel, 1979). It is likely that shivering in LI was the result of significantly lower skin temperatures observed during exposures at 9°C rather than changes in core temperature.

The differences in the change in Tre during light intensity exercise between 9°C and 21°C can be explained by the differences in heat storage of about 30 $W \cdot m^{-2}$. These exposures resulted in differences in heat flow of about 50 $W \cdot m^{-2}$ which were partially offset by the difference in $\dot{M}$ of about 25 $W \cdot m^{-2}$. The difference in heat storage between the two exposures can be additionally attributed to respiratory heat loss. In MI the larger difference in the change in Tre between exposures can be attributed to a larger difference in heat storage of about 45 $W \cdot m^{-2}$. This was due to the inter-trial difference in the rate of heat loss that was not offset by a corresponding difference in $\dot{M}$. Thus, this hypothesis was accepted.

### 6.2 Hypothesis B:

**"Contrasting with what has been reported for males, the increase in $\dot{M}$ seen during exercise in the cold will not be associated with any greater muscle glycogen utilization than is the case when the same exercise is performed at a comfortable temperature."**

As discussed earlier, it has been demonstrated previously that women have a greater tendency toward lipid metabolism than males during rest and exercise. In the current study, glycogen content decreased significantly in MI but not LI but there was no effect of temperature on the extent of the change in glycogen in either group. These results contrast with our earlier work with male subjects (Jacobs et al., 1985), where we observed a 23% decrease in muscle glycogen content in LI at 9°C but no change when the same exercise was performed at 21°C.

In the current study, during light intensity exercise, but not the higher intensity exercise, the higher $\dot{M}$ indicates that more substrates were oxidized to fuel metabolism when the exercise was performed in the cold. Contrasting with

our earlier reports with male subjects, the current data do not suggest that there was a selective increase in carbohydrate oxidation to fuel $\dot{M}$. The results support acceptance of this hypothesis.

## 7. SUMMARY AND CONCLUSIONS

A. Data collection for the second phase of this project was completed in accordance with the experimental protocol. Vasoconstriction and lack of blood flow to the extremities resulted in some difficulty in obtaining venous blood samples during light exercise in the cold.

B. Shivering was induced during rest at 9°C and probably continued during light intensity exercise, which resulted in increases in metabolic heat production. The higher intensity exercise apparently resulted in sufficient heat production to eliminate shivering during exercise.

C. Females used muscle glycogen as one of the carbohydrate energy stores to fuel muscle contraction during exercise, as reported previously for male subjects. Contrasting with males, the increase in metabolic rate seen during low intensity exercise in the cold was not accompanied by an increase in muscle glycogen utilization. During light intensity exercise in the cold, both carbohydrate oxidation and fat oxidation increased to fuel $\dot{M}$. The relative contributions of carbohydrate, fat and protein oxidation to fueling $\dot{M}$ were similar for both 21°C and 9°C trials, regardless of the intensity of exercise. These observations are consistent with a hypothesis that female skeletal muscle is less predisposed than males to selectively "prefer" carbohydrate oxidation to fuel increases in energy metabolic flux.

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Table 1. Physical characteristics of subjects.

| Subject | Age (y) | Height (cm) | Weight (kg) | Fat (%) | BSA ($m^2$) | Oral Contraceptives | W4 (watts) | Exercise Intensity (W) | Group |
|---|---|---|---|---|---|---|---|---|---|
| BN | 28 | 153.8 | 60.2 | 28.9 | 1.58 | none | 101 | 30 | LI |
| EA | 28 | 160.5 | 55.5 | 21.5 | 1.57 | none | 90 | 27 | LI |
| EM | 23 | 171.6 | 63.5 | 22.0 | 1.75 | none | 130 | 39 | LI |
| JV | 19 | 166.3 | 71.5 | 28.0 | 1.79 | yes | 120 | 36 | LI |
| ML | 20 | 157.2 | 50.5 | 23.1 | 1.48 | yes | 70 | 21 | LI |
| PJ | 35 | 168.0 | 62.4 | 31.1 | 1.70 | none | 94 | 28 | LI |
| SP | 29 | 167.0 | 56.7 | 17.3 | 1.63 | none | 154 | 46 | LI |
| SS | 22 | 170.0 | 69.7 | 23.6 | 1.80 | none | 159 | 48 | LI |
| BS | 34 | 170.0 | 73.1 | 30.1 | 1.84 | none | 112 | 67 | MI |
| CM | 19 | 175.6 | 63.8 | 14.3 | 1.77 | none | 177 | 106 | MI |
| CS | 23 | 169.0 | 62.5 | 20.7 | 1.71 | none | 159 | 91 | MI |
| DL | 34 | 163.4 | 60.5 | 24.9 | 1.65 | none | 95 | 57 | MI |
| EI | 23 | 159.0 | 54.0 | 15.0 | 1.54 | none | 88 | 53 | MI |
| LC | 20 | 138.0 | 55.6 | 34.3 | 1.41 | none | 144 | 86 | MI |
| LZ | 20 | 156.9 | 52.7 | 15.0 | 1.51 | none | 129 | 77 | MI |
| MM | 20 | 157.0 | 62.0 | 25.3 | 1.62 | yes | 117 | 70 | MI |
| Mean LI | 25.5 | 164.3 | 61.3 | 24.4 | 1.66 | | 114.8 | 34.4 | |
| ±SD LI | 5.4 | 6.4 | 7.1 | 4.6 | 0.12 | | 31.6 | 9.5 | |
| mean MI | 24.1 | 161.1 | 60.5 | 22.4 | 1.63 | | 127.6 | 75.9 | |
| ±SD MI | 6.3 | 11.5 | 6.6 | 7.5 | 0.14 | | 30.9 | 17.9 | |
| mean All | 24.8 | 162.7 | 60.9 | 23.4 | 1.6 | | 121.2 | 55.1 | |
| ±SD All | 5.7 | 9.2 | 6.6 | 6.1 | 0.1 | | 30.9 | 25.5 | |

Table 2. Day of menstrual cycle and intial rectal temperature before exposures

| Subject | Group | Day of Cycle | | Initial Tre (°C) | |
|---|---|---|---|---|---|
| | | 21°C | 9°C | 21°C | 9°C |
| BN | LI | 15 | 6 | 37.05 | 37.10 |
| EA | LI | 19 | 22 | 37.30 | 37.06 |
| EM | LI | 4 | 18 | 36.90 | 36.80 |
| JV | LI | 13 | 8 | 36.80 | 36.70 |
| ML | LI | 25 | 17 | 37.30 | 37.40 |
| PJ | LI | 6 | 13 | 37.20 | missing |
| SP | LI | 24 | 3 | 37.00 | 37.20 |
| SS | LI | 10 | 24 | 36.80 | 36.70 |
| BS | MI | 12 | 7 | 37.40 | 37.40 |
| CM | MI | 16 | 22 | 36.90 | 36.70 |
| CS | MI | 24 | 12 | 37.50 | 37.10 |
| DL | MI | 14 | 28 | 37.07 | 37.30 |
| EI | MI | 11 | 18 | 37.00 | missing |
| LC | MI | 13 | 19 | 37.00 | 36.90 |
| LZ | MI | 15 | 22 | 36.60 | 36.70 |
| MM | MI | 28 | 4 | 36.80 | 37.10 |
| | | | Mean, LI ± SD | 37.04 0.21 | 36.99 0.27 |
| | | | Mean, MI ± SD | 37.03 0.30 | 37.03 0.28 |

Table 3. Mean core temperature before and during MI or LI exercise at ambient temperatures of 9°C or 21°C.

| Subject | Group | Mean Core Temperature (°C) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 21°C | | | | 9°C | | | |
| | | Rest | Exercise | | | Rest | Exercise | | |
| | | | 10 min | 20 min | 30 min | | 10 min | 20 min | 30 min |
| EA | LI | 37.05 | 37.12 | 37.20 | 37.33 | 37.21 | 36.96 | 36.96 | 37.01 |
| BN | LI | 36.49 | 36.35 | 36.59 | 36.72 | 37.13 | 37.15 | 37.20 | 37.27 |
| EM | LI | 36.87 | 36.40 | 36.19 | 36.14 | 37.06 | 36.59 | 36.70 | 36.79 |
| JV | LI | 36.82 | 37.12 | 37.35 | 37.52 | 37.19 | 37.41 | 37.54 | 37.68 |
| ML | LI | 37.24 | 37.25 | 37.30 | 37.41 | 37.41 | 37.13 | 37.09 | 37.14 |
| PJ | LI | 37.20 | | | 37.31 | 36.92 | 37.07 | 37.23 | 37.27 |
| SP | LI | 37.12 | 37.15 | 37.36 | 37.58 | 37.24 | 37.36 | 37.56 | 37.83 |
| SS | LI | 36.87 | 36.87 | 36.89 | 36.92 | 37.09 | 36.79 | 36.75 | 36.85 |
| BS | MI | 37.08 | 37.15 | 37.54 | 37.76 | 37.45 | 37.52 | 37.76 | 38.04 |
| CM | MI | 36.77 | 36.70 | 36.93 | 37.30 | 36.64 | 36.50 | 36.56 | 36.80 |
| CS | MI | 37.32 | 37.28 | 37.40 | 37.60 | 36.79 | 36.48 | 36.70 | 36.93 |
| DL | MI | 37.02 | 36.64 | 36.97 | 37.23 | 36.89 | 36.62 | 36.86 | 37.11 |
| EI | MI | 36.78 | 36.80 | 36.96 | 37.22 | 37.12 | 37.05 | 36.95 | 37.02 |
| LC | MI | 37.12 | 37.05 | 37.59 | 37.97 | 36.71 | 36.80 | 36.96 | 37.27 |
| LZ | MI | 36.51 | 36.40 | 36.78 | 37.28 | 36.85 | 36.53 | 36.58 | 36.83 |
| MM | MI | 36.78 | 36.72 | 37.12 | 37.40 | 37.10 | 37.15 | 37.21 | 37.38 |
| Mean, LI | | 36.95 | 36.89 | 36.98 | 37.12 | 37.16 | 37.06 | 37.13 | 37.23 |
| SD, LI | | 0.25 | 0.37 | 0.45 | 0.49 | 0.14 | 0.27 | 0.32 | 0.37 |
| Mean, MI | | 36.92 | 36.84 | 37.16 | 37.47 | 36.94 | 36.83 | 36.95 | 37.17 |
| SD, MI | | 0.26 | 0.29 | 0.31 | 0.28 | 0.26 | 0.38 | 0.39 | 0.40 |

Table 4. Mean heat storage during moderate and low intensity exercise at 21 and 9°C (mean ± SD).

| | Mean heat storage ($W \cdot m^{-2}$) | | | |
|---|---|---|---|---|
| | 21°C | | 9°C | |
| Group | Rest | Exercise | Rest | Exercise |
| MI | -40.4 ± 6.7 | 124.4 ± 29.1 | -87.6 ± 11.8 | 78.9 ± 28.5 * |
| LI | -38.3 ± 15.5 | 58.1 ± 15.6 | -82.6 ± 13.3 | 28.9 ± 17.6* + |
| Total | -39.3 ± 12 | 91.3 ± 41 | -85.1 ± 12* | 53.9 ± 35* |

* Significantly different from 21°C
\+ significantly different from MI

Table 5. Mean heat flow during moderate and low intensity exercise at 21 and 9°C (mean ± SD).

| | Mean heat flow ($W \cdot m^{-2}$) | | | |
|---|---|---|---|---|
| | 21°C | | 9°C | |
| Group | Rest | Exercise | Rest | Exercise |
| MI | 71.0 ± 9.3 | 102.0 ± 9.5 | 126.9 ± 7.3 | 148.6 ± 8.8 |
| LI | 72.1 ± 8.8 | 98.6 ± 10.7 | 129.8 ± 4.8 | 150.7 ± 8.1 |
| Total | 71.5 ±9 | 100.3 ± 10 | 128.4 ±6* | 149.3 ± 8 * |

* Significantly different from 21°C

Table 6. Mean skin temperature during moderate and low intensity exercise at 21 and 9°C (mean ± SD).

| Group | Mean skin temperature (°C) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 21°C | | | | 9°C | | | |
| | Rest | Exercise | | | Rest | Exercise | | |
| | | 10 min | 20 min | 30 min | | 10 min | 20 min | 30 min |
| MI | 31.0 ± .7 | 29.7 ± .5 | 29.9 ± .6 | 30.4 ± .8 | 27.5 ± .7 | 25.6 ± .9 | 24.9 ± .9 | 24.9 ± .9 |
| LI | 31.1 ± .7 | 29.9 ± .5 | 30.0 ± .6 | 30.3 ± .7 | 27.9 ± .7 | 25.3 ± .7 | 24.9 ± .8 | 24.9 ± .8 |
| Total | 31.1 ± .7 | 29.8 ± .5 | 29.9 ± .6 | 30.3 ± .7 | 27.7 ± .7 * | 25.5 ± .8 | 24.9 ± .8 | 24.9 ± .8 |

* different from 21°C ($p < 0.001$)

Table 7. Metabolic rate during rest and exercise in LI and MI at 9°C and 21°C.

| Subject | Group | M ($W \cdot m^{-2}$) | | | |
|---|---|---|---|---|---|
| | | Rest | | Exercise (0-30 min) | |
| | | 21°C | 9°C | 21°C | 9°C |
| BN | LI | 43.34 | 46.12 | 159.83 | 176.85 |
| EA | LI | 48.33 | 47.23 | 171.38 | 211.28 |
| EM | LI | 43.69 | 52.08 | 175.52 | 212.87 |
| JV | LI | 69.53 | 89.48 | 211.31 | 221.56 |
| ML | LI | 46.03 | 48.03 | 160.67 | 201.28 |
| PJ | LI | 41.03 | 74.42 | 170.49 | 189.24 |
| SP | LI | 38.52 | 67.30 | 228.64 | 267.52 |
| SS | LI | 50.12 | 64.54 | 197.83 | 194.00 |
| BS | MI | 43.14 | 54.15 | 212.78 | 234.30 |
| CM | MI | 40.92 | 48.12 | 310.63 | 307.57 |
| CS | MI | 51.85 | 43.40 | 275.85 | 267.31 |
| DL | MI | 42.96 | 53.70 | 213.66 | 225.98 |
| EI | MI | 49.92 | 56.30 | 235.42 | 244.90 |
| LC | MI | 43.43 | 73.05 | 309.73 | 298.49 |
| LZ | MI | 47.01 | 47.12 | 286.63 | 287.34 |
| MM | MI | 43.49 | 43.95 | 245.38 | 257.03 |
| | Mean, LI | 47.57 | 61.15 | 184.46 | 209.32* |
| | ±SD, LI | 9.63 | 15.59 | 25.27 | 27.54 |
| | Mean, MI | 45.34 | 52.47 | 261.26 | 265.36 |
| | ±SD, MI | 3.84 | 9.60 | 39.96 | 30.17 |
| | Total, Mean | 46.46 | 93.87* | 222.86 | 237.34 |
| | ± SD | 7.18 | 13.29 | 51.15 | 40.20 |

* Significant difference from 21°C

Table 8. Substrate oxidation rates during rest and either moderate intensity (MI) or light intensity (LI) exercise at 9°C and 21°.

| Subject | Group | RESTING DATA ($J\ s^{-1}$) | | | | | | EXERCISE DATA ($J\ s^{-1}$) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Carbohydrate | | Fat | | Protein | | Carbohydrate | | Fat | | Protein | |
| | | 21°C | 9°C | 21°C | 9°C | 21°C | 9°C | 21°C | 9°C | 21°C | 9°C | 21°C | 9°C |
| | | | | | | | | | | | | | |
| BS | MI | 24.9 | 46.7 | 42.7 | 43.8 | 11.7 | 9.1 | 269.7 | 295.4 | 109.9 | 126.4 | 11.7 | 9.1 |
| CM | MI | 18.0 | 51.9 | 40.2 | 23.1 | 14.3 | 10.3 | 317.7 | 401.3 | 218.6 | 133.6 | 14.3 | 10.3 |
| CS | MI | 17.3 | 41.4 | 63.7 | 27.8 | 7.8 | 5.2 | 241.8 | 320.9 | 222.9 | 131.9 | 7.8 | 5.2 |
| DL | MI | 21.0 | 38.7 | 40.7 | 42.0 | 9.1 | 7.8 | 237.7 | 273.0 | 105.5 | 91.8 | 9.1 | 7.8 |
| EI | MI | 26.2 | 27.8 | 39.0 | 46.0 | 11.7 | 13.0 | 223.8 | 202.1 | 127.2 | 162.2 | 11.7 | 13.0 |
| LC | MI | 35.9 | 72.6 | 18.8 | 27.6 | 6.5 | 2.6 | 312.5 | 219.9 | 116.9 | 197.6 | 6.5 | 2.6 |
| LZ | MI | 32.7 | 23.1 | 30.5 | 28.5 | 7.8 | 19.5 | 251.0 | 314.7 | 174.2 | 99.9 | 7.8 | 19.5 |
| MM | MI | 40.9 | 40.4 | 24.3 | 21.6 | 5.2 | 9.1 | 309.5 | 280.7 | 82.5 | 126.2 | 5.2 | 9.1 |
| BN | LI | 23.4 | 29.8 | 39.7 | 36.4 | 5.2 | 6.5 | 101.7 | 131.2 | 145.1 | 141.1 | 5.2 | 6.5 |
| EA | LI | 47.2 | 31.9 | 22.3 | 35.9 | 6.5 | 6.5 | 218.1 | 218.3 | 44.9 | 107.5 | 6.5 | 6.5 |
| EM | LI | 44.5 | 72.7 | 22.8 | 7.9 | 9.1 | 10.4 | 177.4 | 243.7 | 120.1 | 117.7 | 9.1 | 10.4 |
| JV | LI | 72.8 | 127.6 | 42.6 | 26.1 | 9.1 | 6.5 | 181.3 | 256.2 | 187.9 | 134.1 | 9.1 | 6.5 |
| ML | LI | 42.3 | 28.5 | 17.0 | 32.5 | 9.1 | 10.3 | 151.9 | 226.0 | 77.5 | 62.6 | 9.1 | 10.3 |
| PJ | LI | 27.9 | 34.1 | 34.2 | 87.5 | 7.8 | 5.2 | 124.3 | 152.1 | 158.5 | 165.3 | 7.8 | 5.2 |
| SP | LI | 27.1 | 45.4 | 23.9 | 55.1 | 11.7 | 9.1 | 207.4 | 287.8 | 153.5 | 139.0 | 11.7 | 9.1 |
| SS | LI | 53.4 | 56.9 | 26.6 | 50.3 | 10.3 | 9.1 | 201.7 | 167.5 | 144.5 | 173.0 | 10.3 | 9.1 |
| | | | | | | | | | | | | | |
| mean, MI | | 27.1 | 42.8 | 37.5 | 32.5 | 9.3 | 9.6 | 270.5 | 288.5 | 144.7 | 133.7 | 9.3 | 9.6 |
| SD, MI | | 8.6 | 15.2 | 13.7 | 9.8 | 3.1 | 5.1 | 37.8 | 62.1 | 53.7 | 33.6 | 3.1 | 5.1 |
| | | | | | | | | | | | | | |
| mean, LI | | 42.3 | 53.4 | 28.6 | 41.5 | 8.6 | 8.0 | 170.5 | 210.3 | 129.0 | 130.0 | 8.6 | 8.0 |
| SD, LI | | 16.4 | 33.7 | 9.1 | 23.6 | 2.1 | 2.0 | 41.4 | 54.8 | 46.7 | 34.9 | 2.1 | 2.0 |

Table 9. Muscle glycogen concentrations (mmol glucose•kg dry muscle$^{-1}$) in MI and LI at 21°C and 9 °C.

| Subject | Group | 21°C | | 9°C | |
|---|---|---|---|---|---|
| | | pre | post | pre | post |
| BN | LI | 445.45 | 347.81 | 434.41 | 318.76 |
| EA | LI | 389.14 | 383.88 | 471.15 | 546.90 |
| EM | LI | 313.66 | 316.16 | 415.47 | 399.56 |
| JV | LI | 396.00 | 372.56 | 374.93 | 396.47 |
| ML | LI | 390.64 | 398.76 | 488.64 | 502.22 |
| SP | LI | 448.54 | 398.00 | 499.76 | 405.00 |
| SS | LI | 409.74 | 363.70 | 602.37 | 518.47 |
| BS | MI | 249.91 | 227.38 | 352.20 | 166.72 |
| CM | MI | 380.85 | 279.80 | 367.11 | 315.18 |
| CS | MI | 636.42 | 416.94 | 587.63 | 423.91 |
| DL | MI | 328.94 | 312.00 | 450.88 | 376.13 |
| EI | MI | 329.82 | 253.43 | 207.36 | 193.79 |
| LC | MI | 511.29 | 211.71 | 463.94 | 362.21 |
| LZ | MI | 335.98 | 319.88 | 280.42 | 165.41 |
| MM | MI | 357.91 | 258.64 | 396.85 | 340.08 |
| MEAN, LI | | 399.02 | 368.70 | 469.53 | 441.05 |
| SD, LI | | 45.07 | 29.52 | 73.07 | 82.57 |
| MEAN, MI* | | 391.39 | 284.97 | 388.30 | 292.93 |
| SD, MI | | 123.30 | 65.21 | 116.85 | 102.55 |
| MEAN, TOTAL | | 394.95 | 324.04 | 426.21 | 362.05 |
| SD, TOTAL | | 92.13 | 66.10 | 104.28 | 118.45 |

* Significant pre-post difference in MI group only. No effect of temperature.

Table 10. Changes in plasma volume during each exposure.

| Subject | Group | Changes in blood plasma volume (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 21°C | | | 9°C | | |
| | | Pre exercise | Mid exercise | End exercise | Pre exercise | Mid exercise | End exercise |
| EM | LI | -4.57 | -12.77 | -10.20 | N/A | -13.51 | -15.88 |
| JV | LI | -5.30 | -10.29 | -9.61 | -6.66 | -22.72 | -23.24 |
| SS | LI | -5.77 | -10.13 | -11.36 | -8.19 | -14.95 | -14.71 |
| EA | LI | -5.95 | -16.00 | N/A | N/A | N/A | N/A |
| BN | LI | 3.87 | -2.41 | 0.69 | -12.94 | -11.70 | -17.33 |
| SP | LI | -5.13 | -10.43 | -12.90 | -13.10 | -19.27 | -19.27 |
| PJ | LI | -3.15 | -12.52 | -11.87 | -9.09 | -14.71 | -14.49 |
| ML | LI | -6.11 | -15.76 | -13.73 | -11.70 | -18.20 | -19.02 |
| LC | MI | -5.13 | -19.37 | -19.89 | -15.45 | -20.45 | -21.54 |
| CM | MI | -8.95 | -25.54 | -26.52 | -11.67 | -21.94 | -20.61 |
| MM | MI | -8.13 | -11.59 | -12.21 | -10.43 | -20.22 | -17.68 |
| LZ | MI | -0.75 | -12.70 | -13.89 | -11.63 | | -18.08 |
| EI | MI | -5.51 | -16.58 | -13.97 | -14.14 | -22.32 | -20.45 |
| CS | MI | -7.43 | -16.56 | -17.68 | -2.36 | -17.15 | -14.55 |
| BS | MI | 3.97 | -14.71 | -13.51 | -2.33 | -14.89 | -14.89 |
| DL | MI | -8.26 | -15.08 | -13.04 | -14.29 | -20.55 | -19.44 |
| | Mean, LI | -4.01 | -11.29 | -9.85 | -10.28 | -16.44 | -17.71 |
| | SD, LI | 3.32 | 4.27 | 4.86 | 2.68 | 3.80 | 3.10 |
| | Mean, MI | -5.02 | -16.52 | -16.34 | -10.29 | -19.65 | -18.41 |
| | SD, MI | 4.48 | 4.37 | 4.86 | 5.17 | 2.68 | 2.61 |
| | Mean, total | -4.52 | -13.90 | -13.31 | -10.28 | -18.04 | -18.08 |
| | SD, TOTAL | 3.85 | 4.97 | 5.76 | 4.14 | 3.57 | 2.77 |

Table 11. Changes in hemaglobin during each exposure.

| Subject | Group | Hemaglobin ($g \cdot dL^{-1}$) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 21°C | | | | 9°C | | | |
| | | Pre rest | Pre exercise | Mid exercise | End exercise | Pre rest | Pre exercise | Mid exercise | End exercise |
| EM | LI | 12.6 | 13 | 14 | 13.6 | 13.1 | • | 14.2 | 14.6 |
| JV | LI | 12.5 | 13 | 13.3 | 13.2 | 13.6 | 14.1 | 14.8 | 14.9 |
| SS | LI | 13.6 | 14.2 | 14.4 | 14.6 | 13.1 | 13.6 | 14.2 | 14.4 |
| EA | LI | 12.8 | 13.4 | 14.3 | • | 12.4 | • | • | • |
| BN | LI | 13.2 | 13.6 | 14 | 13.8 | 13.2 | 14.2 | 14 | 14.7 |
| SP | LI | 13.5 | 14 | 14.1 | 14.5 | 12.4 | 13.6 | 14.4 | 14.4 |
| PJ | LI | 12.4 | 12.6 | 13.5 | 13.4 | 12.6 | 13.2 | 13.6 | 13.8 |
| ML | LI | 12.4 | 13 | 13.8 | 13.7 | 12.6 | 13.6 | 14.2 | 14.1 |
| LC | MI | 13.4 | 13.9 | 15.3 | 15.4 | 12.6 | 14 | 14.4 | 14.6 |
| CM | MI | 12.6 | 13.2 | 15.1 | 15.3 | 12.4 | 13.4 | 14.2 | 14.2 |
| MM | MI | 13.1 | 13.8 | 14.1 | 14.2 | 13.1 | 13.5 | 14.4 | 14.2 |
| LZ | MI | 13.2 | 13.3 | 14.4 | 14.6 | 13.4 | 14.2 | • | 14.8 |
| EI | MI | 11.7 | 12.2 | 13.2 | 13 | 11.4 | 12.7 | 13.4 | 13.5 |
| CS | MI | 13.2 | 13.8 | 14.8 | 15 | 14.1 | 14.2 | 15.6 | 15.4 |
| BS | MI | 13.1 | 12.6 | 14.4 | 14.2 | 12.8 | 12.9 | 14.1 | 14.1 |
| DL | MI | 12.5 | 13.2 | 13.8 | 13.7 | 12.6 | 14 | 14.6 | 14.4 |
| | Mean, LI | 12.88 | 13.35 | 13.93 | 13.83 | 12.88 | 13.72 | 14.20 | 14.41 |
| | SD, LI | 0.49 | 0.55 | 0.38 | 0.53 | 0.44 | 0.37 | 0.37 | 0.37 |
| | Mean, MI | 12.85 | 13.25 | 14.39 | 14.43 | 12.80 | 13.61 | 14.39 | 14.40 |
| | SD, MI | 0.56 | 0.60 | 0.69 | 0.82 | 0.79 | 0.58 | 0.66 | 0.56 |
| | Mean, Total | 12.86 | 13.30 | 14.16 | 14.15 | 12.84 | 13.66 | 14.29 | 14.41 |
| | SD, TOTAL | 0.51 | 0.56 | 0.59 | 0.74 | 0.62 | 0.49 | 0.52 | 0.46 |

Table 12. Changes in hematocrit during each exposure.

| Subject | Group | Hematocrit | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 21°C | | | | 9°C | | | |
| | | Pre rest | Pre exercise | Mid exercise | End exercise | Pre rest | Pre exercise | Mid exercise | End exercise |
| BN | LI | 43 | 39 | 41 | 40 | 37 | 41 | 41 | 42 |
| EA | LI | 35 | 36 | 39 | • | 34 | • | • | • |
| EM | LI | 35 | 36 | 37 | 37 | 36 | • | 40 | 40 |
| JV | LI | 34 | 35 | 37 | 37 | 38 | 40 | 41 | 41 |
| ML | LI | 36 | 37 | 40 | 39 | 36 | 39 | 41 | 42 |
| PJ | LI | 37 | 38 | 40 | 40 | 37 | 40 | 42 | 41 |
| SP | LI | 38 | 39 | 42 | 42 | 36 | 39 | 40 | 40 |
| SS | LI | 38 | 39 | 41 | 41 | 36 | 39 | 41 | 40 |
| BS | MI | 36 | 36 | 40 | 40 | 36 | 37 | 40 | 40 |
| CM | MI | 35 | 38 | 42 | 42 | 34 | 37 | 41 | 40 |
| CS | MI | 38 | 40 | 42 | 42 | 40 | 41 | 45 | 44 |
| DL | MI | 36 | 38 | 40 | 49 | 37 | 40 | 42 | 42 |
| EI | MI | 32 | 33 | 36 | 35 | 31 | 34 | 37 | 35 |
| LC | MI | 37 | 38 | 42 | 42 | 34 | 38 | 40 | 40 |
| LZ | MI | 37 | 37 | 40 | 40 | 37 | 41 | • | 43 |
| MM | MI | 38 | 40 | 41 | 41 | 35 | 40 | 43 | 42 |
| | MEAN, LI | 37.00 | 37.38 | 39.63 | 39.43 | 36.25 | 39.67 | 40.86 | 40.86 |
| | SD, LI | 2.83 | 1.60 | 1.85 | 1.90 | 1.16 | 0.82 | 0.69 | 0.90 |
| | MEAN, MI | 36.13 | 37.50 | 40.38 | 41.38 | 35.50 | 38.50 | 41.14 | 40.75 |
| | SD, MI | 1.96 | 2.27 | 2.00 | 3.85 | 2.67 | 2.45 | 2.54 | 2.76 |
| | Mean, total | 36.56 | 37.44 | 40.00 | 40.47 | 35.88 | 39.00 | 41.00 | 40.80 |
| | SD, TOTAL | 2.39 | 1.90 | 1.90 | 3.16 | 2.03 | 1.96 | 1.80 | 2.04 |

Table 13. Changes in blood lactate during each exposure.

| Subject | Group | Lactate (mmol·l$^{-1}$) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 21°C | | | | 9°C | | | |
| | | Pre rest | Pre exercise | Mid exercise | End exercise | Pre rest | Pre exercise | Mid exercise | End exercise |
| BN | LI | 0.76 | 0.69 | 1.29 | 0.99 | 0.75 | 1.34 | 1.63 | 1.66 |
| EA | LI | 0.55 | 0.59 | 1.85 | • | 0.69 | • | • | • |
| EM | LI | 0.18 | 0.37 | 0.89 | 0.82 | 0.28 | • | 1.33 | 1.16 |
| JV | LI | 1.31 | 1.12 | 1.17 | 0.96 | 0.71 | 0.64 | 1.26 | 0.92 |
| ML | LI | 0.88 | 0.85 | 1.82 | 1.65 | 0.72 | 0.73 | 2.77 | 2.9 |
| PJ | LI | 0.62 | 0.57 | 1.12 | 0.95 | 0.53 | 0.64 | 1.48 | 1.17 |
| SP | LI | 0.25 | 0.45 | 1.32 | 1.11 | 0.42 | 0.38 | 2.06 | 1.48 |
| SS | LI | 0.57 | 0.61 | 0.94 | 0.92 | 0.28 | 0.56 | 1.36 | 1.29 |
| BS | MI | 0.49 | 0.55 | 1.88 | 1.49 | 0.54 | 0.54 | 1.84 | 1.71 |
| CM | MI | 0.39 | 0.42 | 1.47 | 1.37 | 0.5 | 0.82 | 1.6 | 1.48 |
| CS | MI | 0.62 | 0.58 | 1.2 | 0.94 | 1.1 | 1.06 | 2.4 | 2.29 |
| DL | MI | 0.56 | 0.57 | 2.11 | 2.21 | 0.52 | 0.57 | 2.78 | 2.21 |
| EI | MI | 0.52 | 0.41 | 2.38 | 1.36 | 0.36 | 0.39 | 2.14 | 1.97 |
| LC | MI | 1.02 | 0.93 | 1.88 | 1.73 | 0.72 | 1.06 | 1.39 | 1.42 |
| LZ | MI | 0.43 | 0.36 | 1.97 | 1.62 | 0.35 | 0.47 | • | 1.88 |
| MM | MI | 0.83 | 0.77 | 1.77 | 1.63 | 0.71 | 0.84 | 2.13 | 2.1 |
| | MEAN, LI | 0.64 | 0.66 | 1.30 | 1.06 | 0.55 | 0.72 | 1.70 | 1.51 |
| | SD, LI | 0.36 | 0.24 | 0.36 | 0.28 | 0.20 | 0.33 | 0.54 | 0.66 |
| | MEAN, MI | 0.61 | 0.57 | 1.83 | 1.54 | 0.60 | 0.72 | 2.04 | 1.88 |
| | SD, MI | 0.21 | 0.19 | 0.37 | 0.36 | 0.24 | 0.26 | 0.47 | 0.32 |
| | Mean, total | 0.62 | 0.62 | 1.57 | 1.32 | 0.57 | 0.72 | 1.87 | 1.71 |
| | SD, TOTAL | 0.29 | 0.21 | 0.45 | 0.40 | 0.22 | 0.28 | 0.52 | 0.52 |

Table 14. Changes in blood glucose levels during each exposure.

| Subject | Group | Glucose ($mg \cdot dl^{-1}$) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 21°C | | | | 9°C | | | |
| | | Pre rest | Pre exercise | Mid exercise | End exercise | Pre rest | Pre exercise | Mid exercise | End exercise |
| BN | LI | 77.19 | 71.74 | 77.72 | 74.51 | 71.91 | 72.09 | 76.49 | 74.38 |
| EA | LI | 77.08 | • | • | • | 70.29 | 72.20 | 75.17 | • |
| EM | LI | 76.03 | 71.66 | 77.16 | 80.72 | 77.00 | • | 77.49 | 73.60 |
| JV | LI | 91.56 | 91.56 | 91.56 | 91.56 | 91.56 | 91.56 | 91.56 | 91.56 |
| ML | LI | 64.88 | 66.99 | 72.09 | 69.98 | 64.35 | 63.12 | 68.93 | 64.35 |
| PJ | LI | 72.27 | 73.32 | 77.72 | 77.54 | 61.01 | 67.17 | 67.87 | 67.34 |
| SP | LI | 80.57 | 78.83 | 83.01 | 105.31 | 70.46 | 75.17 | 86.32 | 89.98 |
| SS | LI | 72.03 | 68.37 | 79.00 | 80.39 | 71.86 | 68.55 | 84.92 | 76.74 |
| BS | MI | 65.76 | 64.71 | 67.34 | 63.48 | 66.64 | 66.11 | 65.76 | 62.95 |
| CM | MI | 68.43 | 69.88 | 72.15 | 65.51 | 71.50 | 51.12 | 72.79 | 72.96 |
| CS | MI | 70.81 | 59.31 | 77.95 | 76.66 | 71.86 | 70.64 | 72.55 | 79.52 |
| DL | MI | 67.17 | 68.22 | 91.08 | 77.01 | 72.09 | 62.77 | 75.96 | 71.91 |
| EI | MI | 71.74 | 68.40 | 67.34 | 65.23 | 59.26 | 53.28 | 65.06 | 62.60 |
| LC | MI | 66.32 | 71.50 | 75.71 | 78.29 | 68.26 | 63.41 | 74.09 | 72.79 |
| LZ | MI | 75.52 | 70.46 | 73.77 | 75.52 | 62.97 | 61.58 | • | 51.47 |
| MM | MI | 68.02 | 64.19 | 67.33 | 71.51 | 74.47 | 68.72 | 73.25 | 63.67 |
| | MEAN, LI | 76.45 | 74.64 | 79.75 | 82.86 | 72.31 | 72.84 | 78.59 | 76.85 |
| | SD, LI | 7.72 | 8.37 | 6.11 | 11.93 | 9.19 | 9.15 | 8.40 | 10.43 |
| | MEAN, MI | 69.22 | 67.08 | 74.08 | 71.65 | 68.38 | 62.20 | 71.35 | 67.23 |
| | SD, MI | 3.28 | 4.07 | 7.99 | 6.08 | 5.18 | 6.90 | 4.22 | 8.76 |
| | Mean,total | 72.84 | 70.61 | 76.73 | 76.88 | 70.34 | 67.17 | 75.21 | 71.72 |
| | SD, TOTAL | 6.84 | 7.32 | 7.52 | 10.63 | 7.49 | 9.48 | 7.54 | 10.47 |

Table 15. Changes in non-esterified free fattty acid concentration during each exposure.

| Subject | Group | NEFA ($mmol \cdot l^{-1}$) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 21°C | | | | 9°C | | | |
| | | Pre rest | Pre exercise | Mid exercise | End exercise | Pre rest | Pre exercise | Mid exercise | End exercise |
| BN | LI | 0.45 | 0.42 | 0.76 | 0.68 | 0.47 | 0.62 | 1.25 | 1.14 |
| EA | LI | 0.34 | 0.43 | 0.67 | • | 0.53 | • | • | • |
| EM | LI | 0.16 | 0.17 | 0.27 | 0.35 | 0.36 | • | 0.30 | 0.40 |
| JV | LI | 0.08 | 0.39 | 0.61 | 0.67 | 0.17 | 0.23 | 0.49 | 0.58 |
| ML | LI | 0.29 | 0.40 | 0.56 | 0.54 | 0.64 | 0.85 | 0.89 | 0.84 |
| PJ | LI | 0.58 | 0.65 | 1.20 | 1.15 | 0.74 | 0.95 | 1.06 | 1.22 |
| SP | LI | 0.38 | 0.37 | 0.39 | 0.41 | 0,30 | 0.52 | 0.57 | 0.45 |
| SS | LI | 0.27 | 0.28 | 0.55 | 0.51 | 0.28 | 0.38 | 0.50 | 0.49 |
| BS | MI | 0.38 | 0.50 | 0.59 | 0.78 | 0.44 | 0.51 | 0.66 | 0.62 |
| CM | MI | 0.45 | 0.63 | 0.72 | 0.69 | 0.24 | 0.39 | 0.40 | 0.43 |
| CS | MI | 0.53 | 0.75 | 1.24 | 1.09 | 0.33 | 0.41 | 0.55 | 0.63 |
| DL | MI | 0.49 | 0.54 | 0.71 | 0.78 | 0.49 | 0.52 | 0.52 | 0.63 |
| EI | MI | 0.33 | 0.52 | 0.59 | 0.57 | 0.57 | 0.67 | 1.16 | 1.54 |
| LC | MI | 0.14 | 0.27 | 0.50 | 0.57 | 0.16 | 0.41 | 0.98 | 0.94 |
| LZ | MI | 0.37 | 0.71 | 1.02 | 1.00 | 0.56 | 0.76 | • | 1.55 |
| MM | MI | 0.38 | 0.45 | 0.58 | 0.58 | 0.40 | 0.45 | 0.76 | 0.86 |
| | MEAN, LI | 0.32 | 0.39 | 0.63 | 0.62 | 0.44 | 0.59 | 0.72 | 0.73 |
| | SD, LI | 0.16 | 0.14 | 0.28 | 0.27 | 0.19 | 0.27 | 0.35 | 0.34 |
| | MEAN, MI | 0.38 | 0.55 | 0.74 | 0.76 | 0.40 | 0.52 | 0.72 | 0.90 |
| | SD, MI | 0.12 | 0.15 | 0.26 | 0.20 | 0.15 | 0.13 | 0.27 | 0.43 |
| | Mean, Total | 0.35 | 0.47 | 0.68 | 0.69 | 0.42 | 0.55 | 0.72 | 0.82 |
| | SD, TOTAL | 0.14 | 0.16 | 0.27 | 0.24 | 0.17 | 0.20 | 0.30 | 0.38 |

Table 16. Changes in plasma glycerol during each exposure.

| Subject | Group | Plasma Glycerol ($mmol \cdot l^{-1}$) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 21°C | | | | 9°C | | | |
| | | Pre rest | Pre exercise | Mid exercise | End exercise | Pre rest | Pre exercise | Mid exercise | End exercise |
| BN | LI | 0.06 | 0.08 | 0.20 | 0.17 | 0.09 | 0.15 | 0.43 | 0.35 |
| EA | LI | 0.06 | 0.07 | 0.16 | 0.10 | • | • | • | • |
| EM | LI | 0.05 | 0.06 | 0.09 | 0.12 | 0.06 | • | 0.10 | 0.12 |
| JV | LI | 0.12 | 0.22 | 0.23 | 0.25 | 0.10 | 0.10 | 0.20 | 0.22 |
| ML | LI | 0.05 | 0.06 | 0.13 | 0.11 | 0.10 | 0.14 | 0.17 | 0.20 |
| PJ | LI | 0.08 | 0.08 | 0.25 | 0.25 | 0.11 | 0.16 | 0.25 | 0.28 |
| SP | LI | 0.05 | 0.07 | 0.13 | 0.14 | 0.07 | 0.10 | 0.16 | 0.15 |
| SS | LI | 0.07 | 0.07 | 0.12 | 0.13 | 0.06 | 0.07 | 0.13 | 0.15 |
| BS | MI | 0.06 | 0.06 | 0.14 | 0.19 | 0.05 | 0.10 | 0.19 | 0.16 |
| CM | MI | 0.08 | 0.08 | 0.16 | 0.23 | 0.06 | 0.06 | 0.10 | 0.12 |
| CS | MI | 0.07 | 0.12 | 0.32 | 0.30 | 0.10 | 0.12 | 0.25 | 0.27 |
| DL | MI | 0.07 | 0.07 | 0.19 | 0.20 | 0.06 | 0.09 | 0.14 | 0.19 |
| EI | MI | 0.07 | 0.08 | 0.16 | 0.19 | 0.09 | 0.11 | 0.27 | 0.33 |
| LC | MI | 0.05 | 0.09 | 0.15 | 0.19 | 0.06 | 0.12 | 0.26 | 0.24 |
| LZ | MI | 0.06 | 0.13 | 0.25 | 0.29 | 0.10 | 0.17 | • | 0.30 |
| MM | MI | 0.12 | 0.13 | 0.12 | 0.12 | 0.09 | 0.12 | 0.23 | 0.24 |
| | MEAN, LI | 0.07 | 0.09 | 0.16 | 0.16 | 0.08 | 0.12 | 0.20 | 0.21 |
| | SD, LI | 0.03 | 0.05 | 0.06 | 0.06 | 0.02 | 0.03 | 0.11 | 0.08 |
| | MEAN, MI | 0.07 | 0.09 | 0.19 | 0.21 | 0.07 | 0.11 | 0.21 | 0.23 |
| | SD, MI | 0.02 | 0.03 | 0.07 | 0.06 | 0.02 | 0.03 | 0.06 | 0.07 |
| | MEAN, TOTAL | 0.07 | 0.09 | 0.18 | 0.19 | 0.08 | 0.11 | 0.21 | 0.22 |
| | SD, TOTAL | 0.02 | 0.04 | 0.06 | 0.06 | 0.02 | 0.03 | 0.09 | 0.07 |

**Figure 1.** Core temperature during moderate (MI) and low intensity (LI) exercise at 9°C and 21°C.

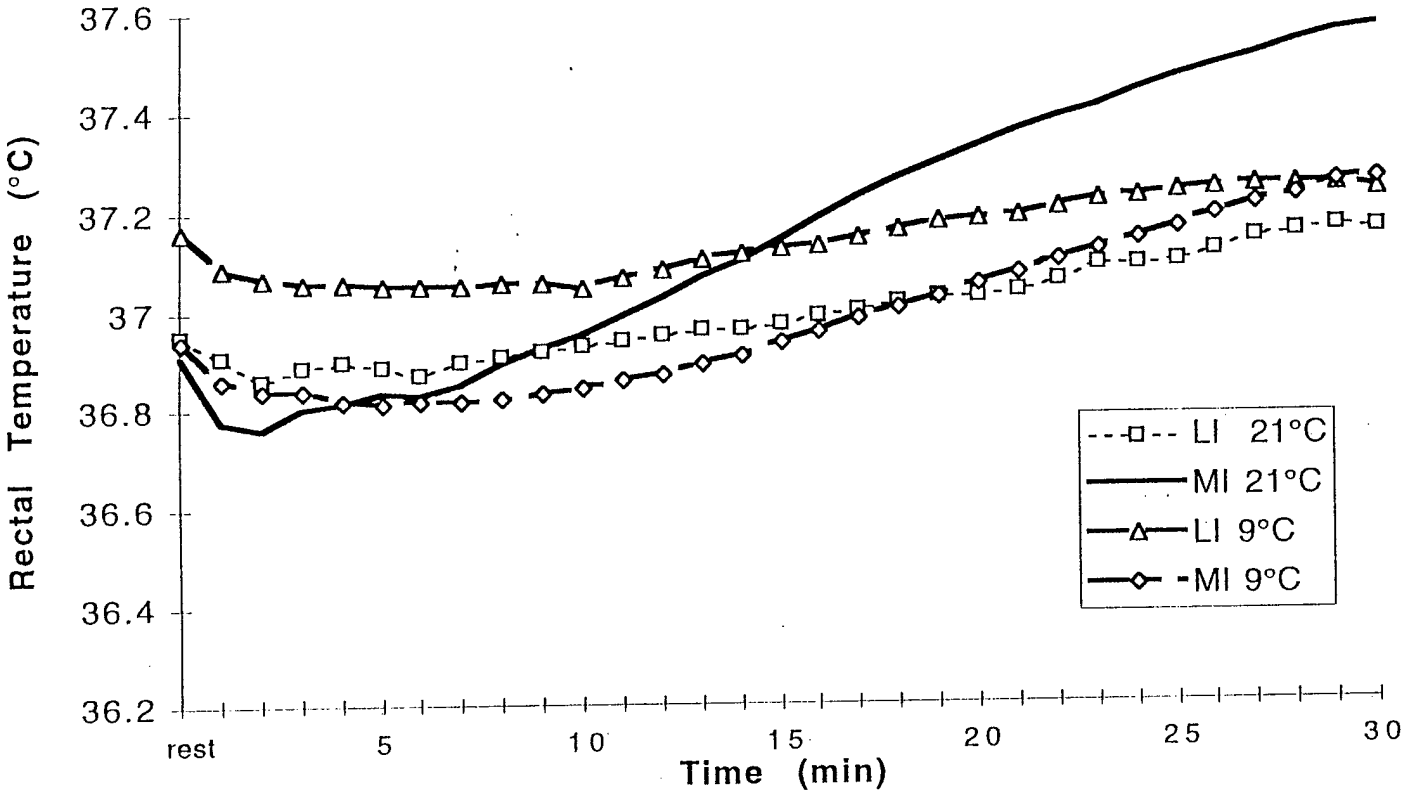


**Figure 2.** Change in rectal temperature at 10, 20 and 30 minutes of exercise during each exposure.

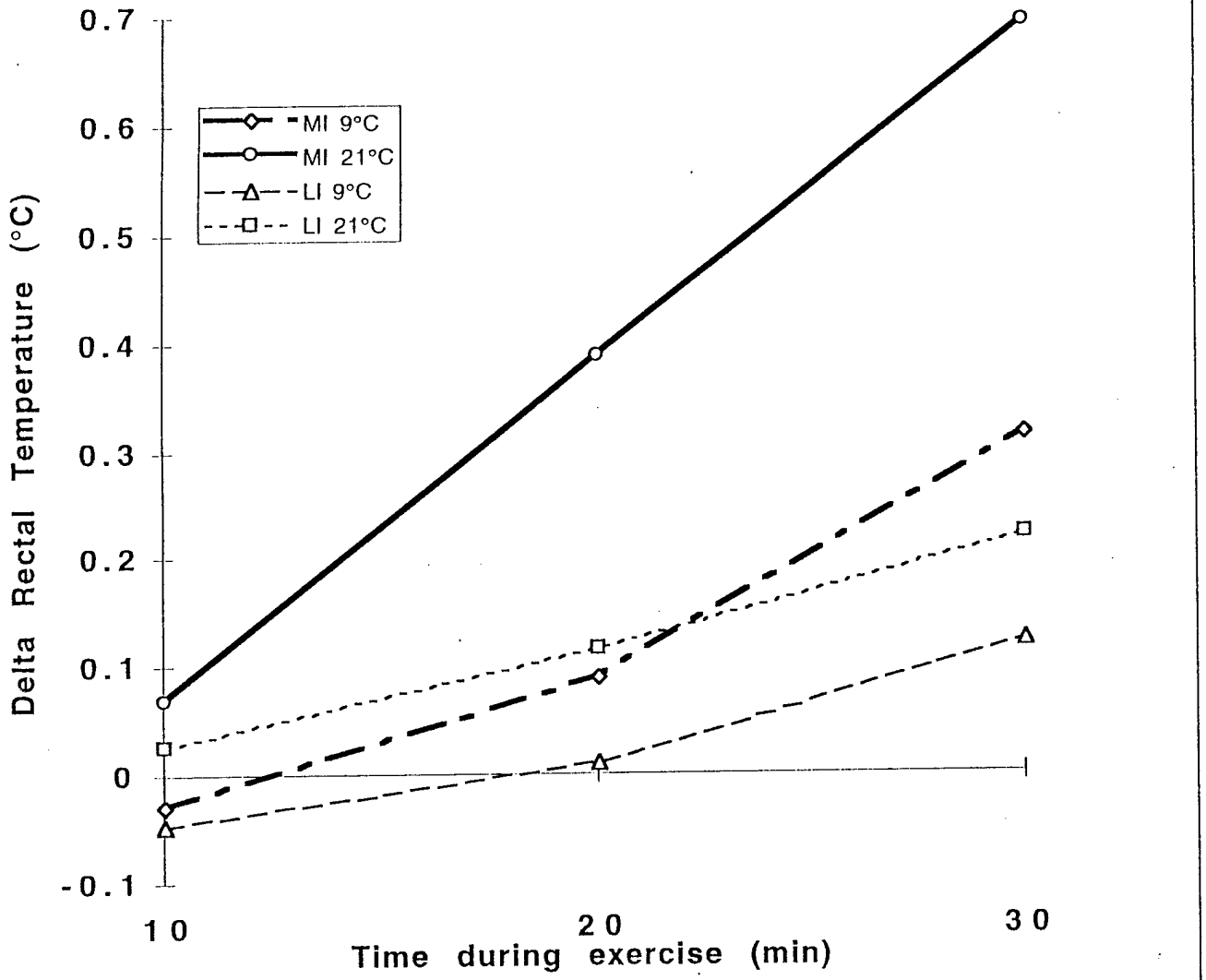


**Figure 3.** Mean metabolic rate during exposures.

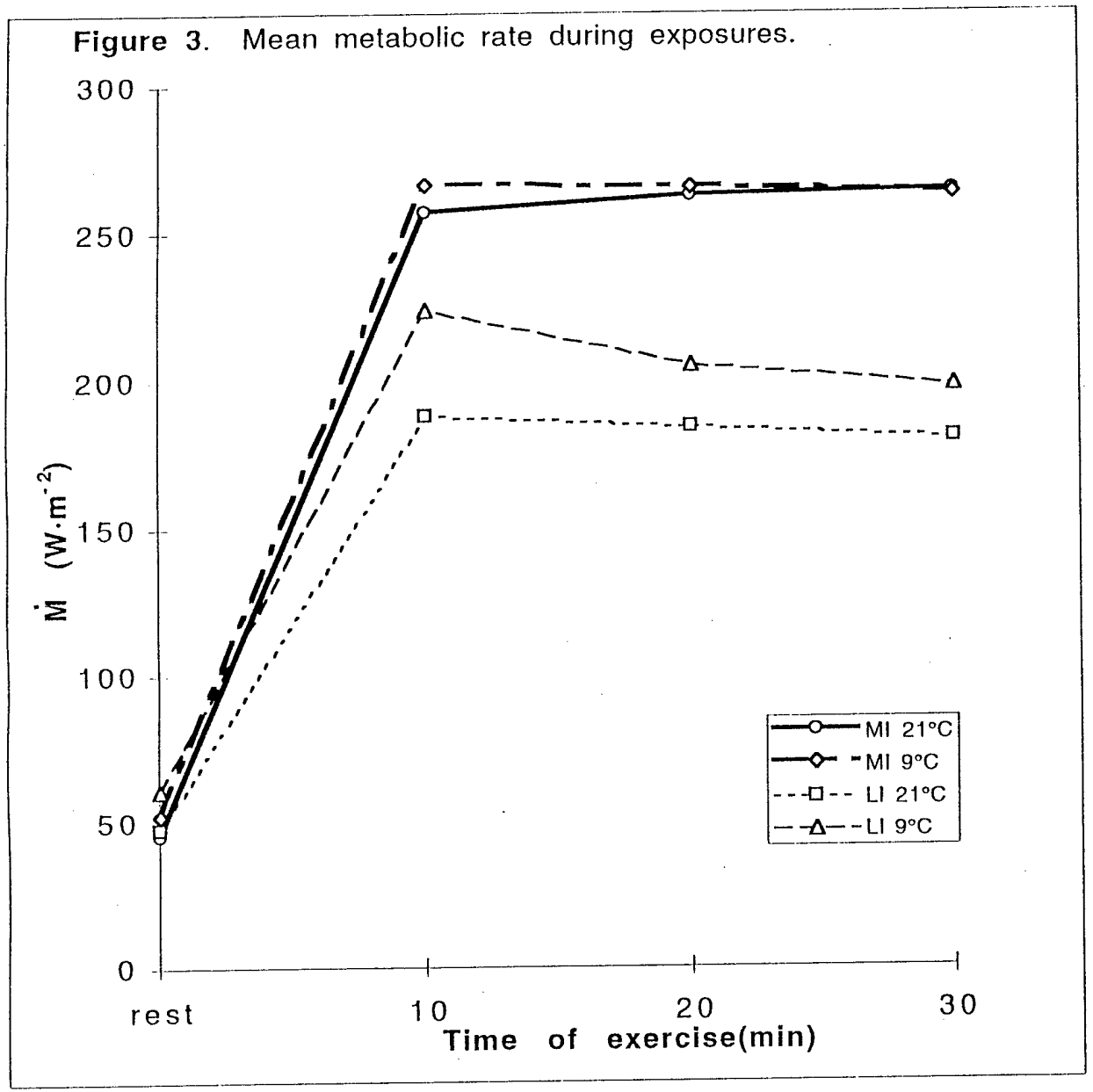


**Figure 4.** Relative contribution of substrate oxidation to M.

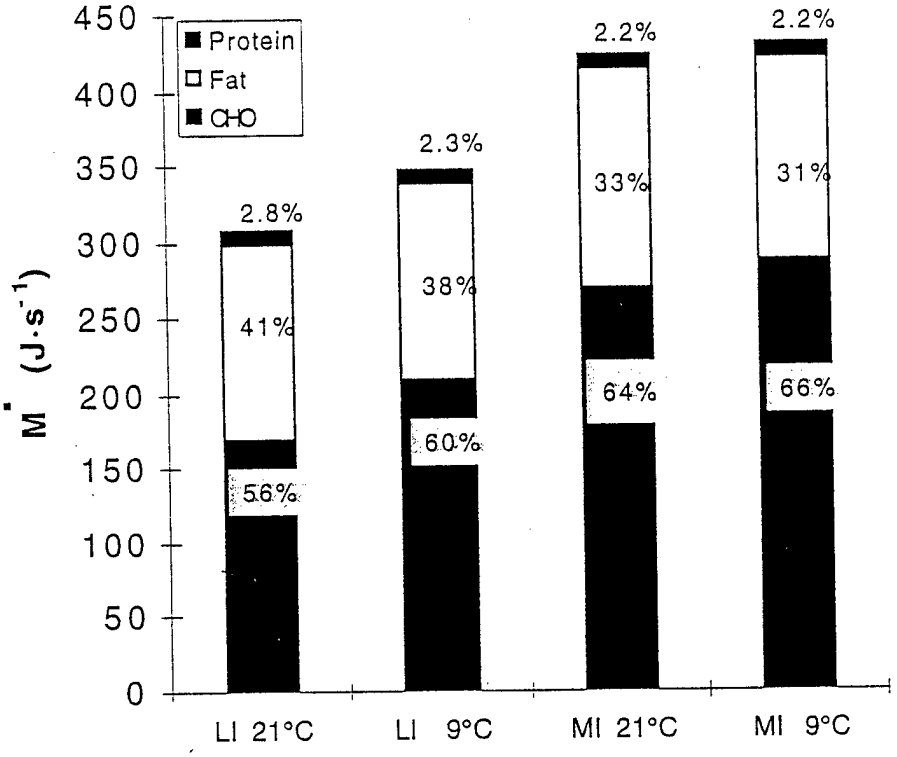
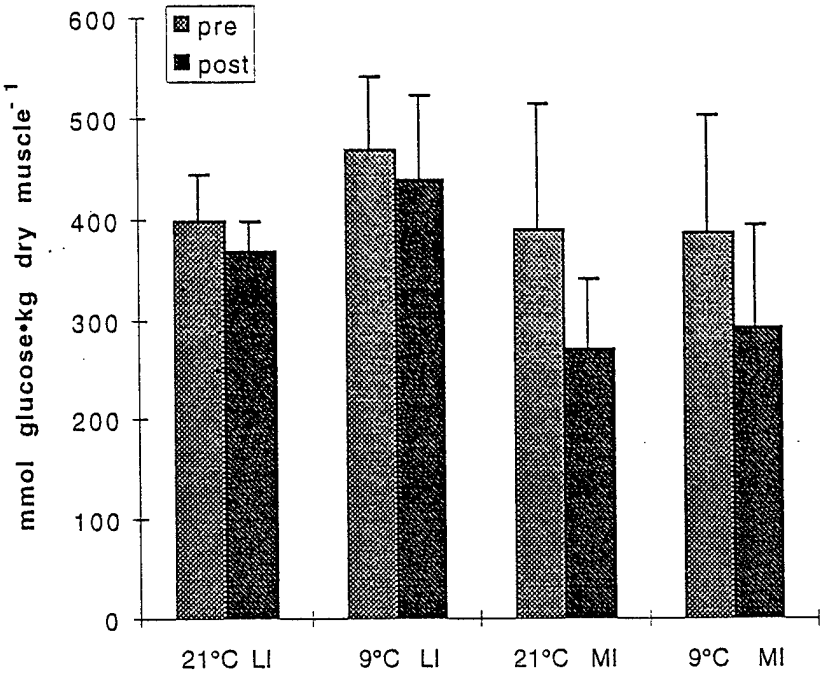


**Figure 5.** Muscle glycogen content during moderate and low intensity exercise at 9°C and 21°C.



# ANNEX C

# ANNUAL REPORT 3

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## 1 ABSTRACT

This document is a progress report which describes the results from the third of a series of studies carried out to clarify the extent of gender-related differences in physiological responses to cold stress, and to evaluate the potential implications for survival time in the cold. One purpose of the study was to document the relative intensity of muscular contraction during shivering in a variety of skeletal muscle groups chosen to represent both central (pectoralis major (PE), rectus abdominis (AB)), and distal (biceps brachii (BB), brachioradialis (BR), rectus femoris (FE), and gastrocnemius (GA)) body regions in order to clarify the extent of the contribution of various body parts to total heat production during shivering. Another purpose was to determine the association in females between the observed increase in muscle electrical activity and the increase in *M*. Fifteen females, (28±6 y; 162.8±4.8 cm; 63.9±8.7 Kg, 23.2±4.7% fat) of normal fitness were recruited. Electromyography was used to monitor muscle electrical activity during a two-hour cold air exposure and the EMG was compared to the EMG that was generated during maximal voluntary contractions of the same muscle groups so that the relative intensity of muscle contraction during shivering could be quantified. Simultaneous measurements of EMG, respiratory gas exchange, body temperatures, and heat flux were used to clarify the association among shivering, *M*, and body temperature regulation. Shivering intensity was greatest in the central versus the peripheral muscles. In fact, AB and PE accounted for 84.5% of whole body measured EMG. There was no significant effect of cold exposure on EMG measured at BR or GA. Shivering intensity did not change during the course of the cold exposure. Similarly, in males, EMG activity was also greatest in the trunk (PE and AB contributing 70.5%). In contrast to females, in males, shivering EMG was significantly higher than pre-exposure in all muscle groups measured and continued to increase throughout the

exposure. Both males and females showed a strong positive correlation between metabolic heat production per lean body mass and whole body shivering.

## 2 INTRODUCTION

Military units operate in cold air and cold water environments, and the associated training or missions can result in personnel being faced with life-threatening situations if they are ill-equipped or unprotected. As demonstrated by the recent winter crash in the Canadian Arctic of a military aircraft carrying infantry personnel, rescue can be delayed for days even when the precise location of survivors is known (de Groot, 1994). Cold water immersion hypothermia recently caused the deaths during training of US Army Rangers (Fort Benning, 1995). In light of such potential emergencies the prediction of survival time (*ST*) in the cold, defined in this document as the elapsed time until the onset of lethal hypothermia, is essential to meet the needs of Search and Rescue authorities. Such predictions are also useful in the analysis of strategic human factors demands of military operations in the cold, to prepare for contingencies of such operations, and to evaluate the potential benefits of equipment/clothing designed to protect the soldier from the cold.

An understanding of *ST* in healthy, sedentary, non-traumatized individuals is based in the following relationships. Once the protective insulation of available shelter or clothing is maximized, cold-stressed humans elevate metabolic heat production ($\dot{M}$) by shivering in an attempt to balance heat loss. Existing models of *ST* in cold air or cold water are based on observations of factors which affect $\dot{M}$ and the rate of heat loss from the body. In such models $\dot{M}$ increases as a function of temperature signals from the core and skin. When cold exposure is too severe for $\dot{M}$ to balance heat loss, *ST* is largely determined by the rate of heat loss from the body. Where there is a balance between $\dot{M}$ and heat loss, *ST* is limited by the endurance time for shivering.

The physiological factors characterizing $\dot{M}$ are relatively complex. Until about a decade ago there was very little empirically based information available in this regard for human subjects. Research has demonstrated that the relationship between

*ST*, $\dot{M}$ and heat loss is affected by the extent of the muscle mass involuntarily recruited during shivering (Bell et al., 1992), connective heat transfer during cold stress (Tikuisis et al., 1991), muscle substrate availability (Jacobs et al., 1994), the type and quantity of substrate oxidized by shivering musculature (Vallerand and Jacobs, 1989), and body composition (Tikuisis et al., 1988). Our research during the last decade has focused on such factors with the objective of generating sufficient knowledge to improve the predictive modeling of *ST* in the cold. A brief review of this research follows.

By measuring the electrical activity of many muscle groups simultaneously during cold-induced shivering, we demonstrated that several large muscle groups are recruited and contract at relatively low intensities that are less than 20% of their maximum force generating capabilities (Bell et al., 1992). Since so many muscle groups are involved in shivering, the sum total of their contractile activities can result in a four or five-fold increase in metabolic rate, and heat production.

Much of our attention has been directed towards the substrates that are used by skeletal muscle to increase heat production during shivering. For example, Vallerand et al. (1988) administered a clinical glucose tolerance test to subjects who were sitting in either cold air or at a comfortable temperature for two hours. These data were the first to show in humans that glucose is eliminated more rapidly from the circulation during cold exposure, presumably to provide more available substrate to fuel the increase in metabolic rate. It is also noteworthy that this more rapid uptake of glucose during cold exposure occurs with lower insulin levels in the cold compared to warm temperatures.

We subsequently continued to attempt to quantify the rates of substrate oxidation of fat, carbohydrate and protein in humans during cold exposure with indirect calorimetric techniques. As one might presume, the increase in metabolic rate during shivering is caused by increases in oxidation of both fat and carbohydrate, but the relative increase in the rate of substrate oxidation caused by shivering is greatest for carbohydrates (Vallerand and Jacobs, 1989). In resting

subjects exposed to either cold air or cold water, carbohydrates and fat contribute approximately equally to heat production (Martineau and Jacobs, 1991; Vallerand and Jacobs, 1989). From a strategic point of view, this finding seems unfortunate because the body's availability of carbohydrates is quite limited compared to the abundant fat and protein stores. We were already aware of the well established positive relationship between muscle glycogen concentration and endurance exercise performance of skeletal muscle and speculated that there may be a similar detrimental effect caused by muscle glycogen depletion on another form of muscle contraction, i.e. shivering and the associated heat production.

We therefore carried out a series of studies on male subjects immersed in 18°C water. The subjects were removed from the water when their rectal temperature reached 35.5° C. Biopsies were taken from the thigh muscle before and after the immersion to evaluate the changes in glycogen as a result of the water immersion (Martineau and Jacobs, 1988). In another study muscle glycogen concentrations were manipulated prior to water immersion by appropriate dietary and exercise protocols (Martineau and Jacobs, 1989); the purpose of these studies was to evaluate the effects of very low and very high glycogen levels on metabolic heat production during the water immersion.

Metabolic rate during cold water immersion, expressed as oxygen consumption, increases to values that are usually around 4 or 5 times normal resting metabolic rate. Infrequently we have observed individuals who exhibit somewhat higher values, 6– or 7 times resting values. Our initial studies suggested that part of this increase in metabolic rate is fueled by muscle glycogen, as all of the subjects demonstrated a decrease in leg glycogen concentration after the water immersion (Martineau and Jacobs, 1988). The second objective of these experiments was to evaluate the effects of manipulating the pre-immersion glycogen levels on heat production during cold water immersion. Our manipulations did result in the subjects entering the water on one trial with muscle glycogen levels that were only about 50% of normal, and on another trial when they were about 150% of normal

(Martineau and Jacobs, 1989). The oxygen consumption during the water immersion, was about the same on each trial. The respiratory exchange ratio (RER), however, differed between trials as expected. Metabolic heat production is calculated based on the combination of RER and oxygen consumption. We observed significantly less metabolic heat production per unit time when the body's carbohydrate stores were depleted compared to the other trials (Martineau and Jacobs, 1989). There was also a significantly more rapid body cooling rate, as reflected by the changes in rectal temperature, when the body had little glycogen stored in its muscles, and presumably also in the liver.

These examples of some of our initial studies were done on subjects resting in cold air or cold water. In light of these findings we hypothesized that the requirement to do physical work superimposed on that cold stress might induce a more rapid breakdown of muscle glycogen than if the same work were done at a comfortable temperature. We therefore had subjects performing either light or heavy exercise once at 9°C air and again on a separate day at 21°C (Jacobs et al., 1985). We found that significantly more glycogen was in fact utilized to do the light exercise in the cold compared to doing the same work at 21°C. There was no difference in glycogen depletion rates, however, for the higher exercise intensities, and this is consistent with earlier observations that the heat production associated with hard exercise is sufficient to offset heat loss to the environment, thus obviating the need for shivering (Hong and Nadel, 1979).

We also carried out investigations of the effects of manipulating the body's circulating fat pools on heat production during cold water immersion. Vallerand and Jacobs (1990) reported that triglycerides infused intravenously were not eliminated more rapidly from the circulation during cold air exposure than during warm air exposure, contrasting with the results for glucose infusion (Vallerand et al., 1988). In another series of experiments, the circulating free fatty acid concentration was manipulated by having our subjects ingest nicotinic acid in the form of niacin pills prior to and during the water immersion (Martineau and Jacobs, 1989b). The effect of

the nicotinic acid is to block lipolysis and this effect is demonstrated by the observation that the plasma free fatty acids and glycerol levels were dramatically reduced prior to, and during, the water immersion. Again contrasting with the effects of manipulating the carbohydrate stores, metabolic heat production was virtually unaffected; the proportion of the total heat production that could be attributed to fat oxidation was significantly reduced, but there was compensation by simply increasing carbohydrate oxidation.

For reasons that are still unclear, carbohydrates seem to be a somewhat preferred substrate during shivering thermogenesis. There are similarities to hard physical exertion in that the body is not able to maintain the same intensity of exertion when carbohydrate stores are depleted, i.e. a shift to a greater reliance on fat oxidation to fuel muscle contraction is not sufficient for the musculature to be able to maintain a high level of exertion, just as body temperature could not be maintained as well when carbohydrate stores were depleted (Martineau and Jacobs, 1989a). We must mention that similar experiments were carried out at USARIEM and they did not detect any significant muscle glycogen utilization during cold water immersion (Young et al., 1989); we can not explain the discrepancies between our studies other than to suggest that perhaps the fact that our subjects were much leaner than those of Young et al. (1989) may be important in this regard.

Gender differences in response to cold stress have been the topic of a limited number of investigations and reviews (Stephenson and Kolka, 1993; Nunneley, 1978; Hayward et al., 1975). It was reported that women cool faster than men during cold water immersion (Kollias et al., 1974; McArdle et al., 1984; Hessemer and Brück, 1985), and this is somewhat surprising considering the greater body fat content of the average female. Body temperature changes associated with the menstrual cycle (Graham et al., 1989), cardiovascular responses to rest and exercise (Stevens et al., 1987; Wagner and Horvath, 1985a,b) are other factors with associated gender differences in response to cold stress. To date potential gender-related physiological differences in responses to cold have not been considered in systematic studies such

as those described above, i.e. quantification of the substrates used to fuel $\dot{M}$ during cold stress, nor in the development of *ST* predictive models, including our own [Tikuisis, 1989; Tikuisis et al., 1988). Specifically, there are established gender differences in the ratio of lean body mass to total body mass and in the proportion of energy derived from carbohydrate or fat metabolism during exercise (Tarnopolsky et al., 1990). There are, however, studies of gender differences with regard to skeletal muscle metabolism during exercise which suggest that untrained female musculature has an enzymatic profile which is predisposed to greater dependency on lipid metabolism than male muscle tissue (Green et al., 1984). In male and female subjects matched for their physical training status, exertion at the same relative intensity is fueled by carbohydrate oxidation to a greater extent in males, and by lipid oxidation to a greater extent in females (Tarnopolsky et al., 1990; Phillips et al., 1993; Tarnopolsky et al., 1995). Although potentially advantageous for endurance exercise, the evidence presented above relating to the importance of carbohydrate oxidation for shivering thermogenesis suggests that less carbohydrate oxidation may be disadvantageous in terms of *ST* in the cold. However, even if the magnitude of the increase in $\dot{M}$ may be less in females than males, the metabolic predisposition favoring lipid oxidation suggests that temperature regulation may not be as negatively influenced when glycogen availability is compromised. In terms of the muscle mass involved in shivering, models of human thermoregulation during cold stress use a fixed value to represent the contribution of the musculature of various body segments to the increase in $\dot{M}$ due to shivering. For example, this constant for the contribution of the trunk has previously only been estimated and ranged from 55-85% (Montgomery, 1974; Stolwijk, 1970; Hancock, 1980). We recently experimentally determined this value for male subjects to be 71% (Bell et al., 1992), but here again no data are yet available for female subjects. The implications of these gender differences, if they apply to cold-induced increases in $\dot{M}$, are potentially of sufficient magnitude to warrant their consideration in a model of *ST* in cold stressed females.

Objectives

This report is a progress report which describes the results from the third of a series of studies carried out to address the issues raised above. The objective was to document the intensity of muscular contraction during shivering in a variety of skeletal muscle groups chosen to represent both central and distal body regions. This information was needed to clarify the extent of the contribution of various body parts to total heat production during shivering. Electromyography (EMG) was used to monitor muscle electrical activity during a two hour cold air exposure and the EMG was compared to the EMG that was generated during maximal voluntary contractions of the same muscle groups so that the relative intensity of muscle contraction during shivering could be quantified. Simultaneous measurements of EMG, respiratory gas exchange, body temperatures, and heat flux were used to clarify the association among shivering, $\dot{M}$, and body temperature regulation. Identical experiments were carried out on male subjects in a study approved as DCIEM protocol #199 in the summer of 1988, by Tikuisis, Bell, and Jacobs, and entitled "The contribution of various muscles to shivering in cold-exposed man."

### 2.1 Hypotheses/Purposes

This study is descriptive thus no experimental hypotheses were postulated *a priori*. One purpose of the study was to document the relative intensity of muscular contraction during shivering in a variety of skeletal muscle groups chosen to represent both central and distal body regions. Another purpose was to determine the association between the observed increase in muscle electrical activity and the increase in $\dot{M}$.

## 3 METHODS

### 3.1 Experimental Design

The protocol and methodology were chosen to enable comparison with data collected for male subjects using a similar protocol (Bell et al. 1992). To facilitate these comparisons we restricted our metabolic studies to the use of indirect calorimetry. Analysis and treatment of EMG data were also similar. Sixteen female subjects, aged 19-37, were recruited from local universities and within our research facility. Subjects did not donate blood for 30 days prior to or during participation in this study.

Subjects reported for their first visit having read a detailed information summary about all aspects of the study. They were given an opportunity to ask questions of the medical officers. Subjects then signed an informed consent and underwent a medical screening. Once receiving medical clearance, physical characteristics including height and weight were determined and percent body fat was estimated after determination of body density by hydrostatic weighing. Maximal oxygen uptake was determined during an exercise test to exhaustion on an electrically braked cycle ergometer. Subjects began cycling at 60 Watts and intensity increased in a ramp fashion by 20 watts every min. Metabolic measurements were made continuously throughout the test as described below. Subjects then performed maximal voluntary contractions with each muscle group as they would on the day of the experiment (see below) in order to familiarize the subject with the procedures and muscle contractions required for MVCs.

### 3.2 Standardization of menstrual and diurnal cycles

All subjects were tested in the follicular phase of their menstrual cycle. The subjects were tested at the same time of day to avoid possible diurnal effects. They were asked to abstain from alcohol for 48 hours before a trial, not exercise within 24 hours of a trial, and fast for 12-14 hours before each trial.

### 3.3 Exposure

On the day of each exposure the subjects reported to the lab in a 12-h post absorptive state, clad in a two piece swimsuit or similar attire. Each experiment consisted of a single exposure to 10°C air at about 40% relative humidity for 2 h. Subjects were asked to inform us on the first day of their menstrual cycle so that their experimental session could be scheduled to occur during the follicular phase. Before experiments, each subject was fitted with EMG electrodes on one side of their body to monitor the EMG of the muscles described below. The other half of their body was be instrumented with 12 heat flow transducers for both heat flow and skin temperature measurements. The subjects also inserted a rectal thermistor, and an intravenous catheter was inserted into an antecubital vein.

*EMG.* The EMG electrodes were placed 0.03 cm apart on the mid-belly of six muscle sites representing the trunk and limbs: pectoralis major (PE), rectus abdominis (AB), biceps brachii (BB), brachioradialis (BR), rectus femoris (FE), and gastrocnemius (GA). The signals were amplified 2000 times (1000 times at the pre-amplifier and twice at the unit) filtered with a band width of 8-500 Hz, integrated, and averaged every 100 ms (ME-3000P-8, Biomation, Almonte, Ontario, Canada). The subject performed a series of voluntary isometric contractions with each muscle so that the regression of EMG against force could be plotted and calculated for each subject. Force was measured using a 0-1000lb load cell (LPT-1000, BLH Electronics Canada, Toronto, Ontario, Canada) and displayed (LCP-100, BLH Electronics Canada, Toronto, Ontario, Canada) such that the subject could see the digital output in kilograms. The series consisted of three consecutive 5-8 s contractions at different levels of force. The time between each contraction was approximately 1 min. Subjects performed a maximal voluntary contraction (MVC) three times. Of the three MVC values, the one corresponding to maximal force also elicited the highest EMG. Subjects were then asked to produce contractions at 50, 20, and 10% and 5% of the highest MVC.

After these measurements the subjects, wearing only a two-piece bathing suit or similar attire, assumed a standard supine position with arms and legs spread apart

on a rope mesh cot and were wrapped in blankets to maintain thermal comfort in a thermal neutral room (21°C). During 15 to 25 min of this 30 min period, the subject's resting metabolic rate, core temperature, skin temperature and heat flow were determined. At the end of this period, the subject was wheeled into the cold chamber, blankets were removed, and the 2-h cold exposure began. Core temperature, heat flow, skin temperatures and EMG activity were recorded continuously and respiratory gas exchange variables were recorded during the periods 5-30, 40-60, 70-90, and 100-120 min. The subject was asked to refrain from voluntary movements except during 1 min stretch breaks allowed every 15 min.

### 3.4 Blood sampling

The protocol called for venous blood samples to be obtained from an antecubital vein just before and after the 30 min rest and immediately post exposure. Difficulties in obtaining sufficient volume of blood were sometimes encountered during the cold exposure, probably due to the combination of vasoconstriction and decreased blood flow to the forearm. A heparin lock (10 U/mL) with the 20 gauge 1 inch catheter was used. A water-proof dressing (Tegaderm®) was placed over the site where the catheter pierced the skin to help stabilize the catheter. Ten mL blood samples were drawn and divided into 4 tubes which were kept on crushed ice: 5 mL were expelled into a tube treated with EGTA (90 mg/mL) and glutathione (60 mg/mL), centrifuged and the plasma was frozen for subsequent determination of catecholamines; 5 mL were dispensed into a chilled, EDTA-treated tube (50 µL were dispensed into tubes containing $HClO_4$ for the subsequent determination of glucose and lactate; samples were taken to determine hematocrit and hemoglobin; the remainder was centrifuged and aliquots of the plasma were subsequently used for the determination of NEFA and glycerol). All samples were stored at -20°C until frozen and then stored at -70°C until assayed.

### 3.5 Biochemistry

Hematocrit was determined by centrifugation (Autocrit Ultra3 centrifuge, Becton-Dickenson, Franklin Labs, New Jersey, U.S.A.). Commercially available kits were used to measure concentrations of free fatty acids (WAKO( NEFA kit, Texas). Glucose and hemoglobin were assayed using automated spectrophotometric techniques (Hemocue, Drew Medical, Mississauga, Ontario, Canada). After deproteinization samples were analyzed for glycerol (Boobis and Maughan, 1983) and lactate (Maughan, 1982). Plasma epinephrine and norepinephrine levels were measured using negative ion chemical ionization gas chromatography-mass spectrometry (Zamecnik, 1997). Changes in plasma volume were calculated from the changes in hematocrit and hemoglobin concentration (Dill and Costill, 1974).

To facilitate calculations of protein oxidation during exposure, the subjects were asked to collect urine for 24 h beginning the morning of, and prior to, the rest and exercise in the environmental chamber. The urine was subsequently assayed for its urea nitrogen concentration (Sigma Kit 640, Sigma Chemicals Co., MO, USA). Analysis of these data are not complete and will be reported in the final report.

### 3.6 Temperature measurements

During 15 to 25 min of the rest period and continuously throughout the cold exposure, the following were measured with an automated data acquisition system and averaged each minute: rectal temperature (Pharmaseal® 400 Series, Baxter Healthcare Corporation, California), mean skin temperature and mean skin heat flow using a 12-point area-weighted system as described elsewhere (Vallerand et al., 1989). For measurement of skin temperature and heat flow, the same twelve, calibrated heat flow sensors (Concept Engineering, model FR-025-TH44033-F8-F. Connecticut) were used throughout the entire experiment.

### 3.7 Respiratory gas exchange measurements

Respiratory gases were monitored using a semi-automated metabolic cart system between 15 and 25 min of the 30 min rest period and continuously throughout the exposure. For this purpose the subject was connected to a mouth-piece, breathing valve, and hose, which directed the expired gases to a 5 litre mixing box, which was connected in series to a ventilation module which measured expired ventilation rate (VMM Ventilation Measurement Module, Interface Associates, Irvine, California). A sample line directed gases from the mixing box to oxygen (AMETEK Model S-3A11, Applied Electrochemistry, Paoli, Pennsylvania) and carbon dioxide (AMETEK Model CD-3A, Applied Electrochemistry, Paoli, Pennsylvania) analyzers. Custom designed computer software (DCIEM/HPP Metabolic Measurement System V1.0, Keefe and Pope, 1997) was used to register the data each minute, and to convert the values into STPD units of oxygen consumption and carbon dioxide production.

### 3.8 Calculation of metabolic heat production

Metabolic heat production rates ($\dot{M}$) were calculated from the respiratory gas exchange measurements of oxygen consumption, carbon dioxide production, and the respiratory exchange ratio (RER) according to Pérronet et al. (1991).

### 3.9 Treatment of data

Integrated EMG data were averaged over each minute. Four minute intervals around the stretch break were not included in the analysis. Resting EMG was subtracted from all values to provide only the non-resting portion of the EMG. Therefore, in this report, EMG refers only to the non-resting component of EMG activity. In order to determine shivering intensity, EMG during shivering was expressed as a percentage of the EMG during the highest MVC. Since the relationship between EMG and force is often non-linear (Bigland-Ritchie, 1981; Bell et al. 1992), a separate calibration factor was applied. These factors for each subject were obtained from a linear fit of the EMG-force relationship of each muscle between

0 and 20% MVC with the assumption that the type of contraction during shivering is the same as that during an isometric MVC. Thus, EMG multiplied by this calibration factor indicates the EMG for the corrected amount of force that is produced relative to the MVC of the muscle and is referred to as EMG(%MVC). A whole body index of shivering intensity (SUM) was determined by multiplying EMG(%MVC) by the relative mass of each respective muscle.

$$SUM = \sum_{i=1}^{6} m_i \bullet EMG(\%MVC)_i \qquad (1)$$

where $m_i$ is the muscle mass fraction of the body represented by muscle *i*. Composition analysis of cadavers (Clarys and Marfell-Jones, 1986) was used to determine limb muscle mass factors and the trunk muscle mass factors were obtained from two anthropometric studies (McConville et al. 1980, Snyder et al. 1975). Muscle mass factors were 0.34 for PE (upper trunk), 0.19 for AB (lower trunk), 0.06 for BI (upper arm), 0.035 for BR (lower arms), 0.29 for FE (upper legs) and 0.085 for GA (lower legs). Finally, a specific muscle's contribution to overall body shivering (%Contribution) was determined by dividing the relative shivering intensity of that muscle by SUM.

$$\%Contribution_i = \frac{m_i \bullet EMG(\%MVC)_i}{SUM} \qquad (2)$$

### 3.10 Statistical analyses

The effect of exposure on in the cold, data were analyzed using a repeated measures analysis of variance. Unless otherwise noted, data are presented as mean values ± standard deviation. It was decided *a priori* that statistical significance would be accepted at the 95% confidence level.

## 4 RESULTS

## 4.1 Subject attrition

Sixteen subjects signed consent forms and completed all familiarization and medical screening procedures. All subjects completed the experiment. EMG data was successfully obtained in only 15 subjects. Since all other data were related to the EMG data, only the data from these fifteen subjects were inlcuded in this report.

## 4.2 Subject characteristics

The physical characteristics of the subjects are presented in **Table 1.** Subjects had a mean age of 28±6.3 and were of average height (162.8±4.8) and weight (63.9±8.7). The mean relative body fat mass was normal (23.2±4.7%). The subjects were of average fitness (mean $\dot{V}O_2$max of 36.9±7 ml/kg/min). Only two subjects took oral contraceptives (Triphasal 21). All subjects were tested in the follicular phase of their menstrual cycle (days 4-10).

## 4.3 Shivering intensity

Individual shivering intensity data expressed as EMG (%MVC) are provided in **Tables 2 and 3 and Figure 1** shows the mean shivering intensity of each muscle. Shivering intensity during the first 90 min of cold exposure increased ($p<0.05$) in all muscle groups except GA and BR. EMG (%MVC) was greater in the central muscles, AB and PE, compared to the peripheral muscles. Averaged over the 2-hour cold exposure, shivering EMG in AB and PE were 13.4 %MVC and 10.4 %MVC, respectively, compared to 2.15 and 1.1 %MVC for FE and BI, respectively. Mean EMG(%Contribution) is presented in Figure 2 and individual data is provided in **Tables 4 and 5.** Each of the peripheral muscles contributed less than 6% to the total EMG compared to PE and AB EMG which contributed 48±28 and 36±27%, respectively. There was not significant difference between PE and AB, however, these two muscles contributed significantly more to total EMG than any of the

peripheral muscles ($p<0.001$). There was no significant change over time of exposure in the %contribution of a given muscle to total EMG activity.

**Table 6** provides the results of the determination of whole body index of shivering (SUM). Mean SUM increased with time ($p<0.001$) until 90 min and then levelled off.

### 4.4 Metabolic heat production

The rate of metabolic heat production ($\dot{M}$) for each subject during exposure is provided in **Table 7**. **Figure 3** illustrates the mean $\dot{M}$ during the exposures. During the rest period, $\dot{M}$ was 37.6±4.5 $W \cdot m^{-2}$. During cold exposure, $\dot{M}$ increased significantly during the first 30 min (to 57.7±12 $W \cdot m^{-2}$) and again from 60 to 90 min. This is consistent with the findings in whole body shivering index (SUM) as described above. Furthermore, if delta $\dot{M}$ (the metabolic heat production during shivering minus resting metabolic heat production) per Kg LBM is regressed against SUM (**Figure 4**), the slope is 4.54 and r=.656 indicating a strong relationship between shivering intensity and metabolic heat production.

### 4.5 Temperature measurements

The individual core temperature responses for 13 subjects are shown in **Table 8** and the mean response is shown in **Figure 5.** Data were unavailable for two subjects due to problems with the rectal thermistor. There was a significant transient rise in Tre during the first 45 min ($p<0.001$) which slowly decreased during the last hour. However, Tre at 120 min was still greater than during pre-exposure ($p<0.001$). Mean pre-exposure core temperature was 36.9±.15°C and at 120 m of cold exposure it was 37.1±.2°C.

Increased shivering was also reflected in the heat transfer coefficient (h) of the body surface. In this study, h was determined as follows:

$$h = \frac{HF}{Tsk - Ta} \qquad (3)$$

where Tsk is the skin temperature at the muscle site and Ta is the ambient temperature. Ambient air conditions including temperature and circulation, did not change during the exposure. Therefore, the increased h must be attributed to the increased movement of the shivering muscle and resulting decrease in the boundary air layers at that site. Heat transfer coefficient was determined for a peripheral site (GA) and a central site (PE), the results of which are provided in **Table 9**. Values of h were calculated during the first 5 min of exposure and during 30 min intervals during the exposure. The 5 min value acts as an initial measurement for the purpose of comparison. A pre-exposure measurement could not be calculated accurately because the exact ambient temperature under the blankets was unknown. Mean h during exposure was greater for PE (7.9±1.8) than GA (5.05±.63) which is consistent with the higher EMG at PE. There was an increase in h throughout the exposure for GA ($p<0.0001$) but no significant change for PE.

### 4.6 Blood metabolites and hormones

Blood metabolite and hormone levels for each subject during each exposure are shown in **Table 10** . As described previously, some difficulties in drawing blood samples in the cold air were encountered and therefore, some post exposure data are missing. Only those subjects for whom both samples were available were included in the statistical analysis. Hemoglobin (Hgb) increased ($p<0.0001$) by 11.5% during cold exposure. Hematocrit (Hct) increased ($p<0.0001$) by 12.4%. Lactate increased 96.8% ($p<0.0003$) during cold exposure while the 2.1% drop in glucose was insignificant. NEFA and glycerol increased by 98.2% and 216.7%, respectively ($p<0.0001$).

These changes in blood metabolite and hormone concentrations should be considered in light of the hemoconcentration (plasma volume decreased 15.44%). However, changes in lactate, NEFA and glycerol were too large to be attributed only to hemoconcentration.

Catecholamine levels have not yet been analyzed but will be included in the Final Report.

## 5 DISCUSSION

This document is a progress report describing the results of the third in a series of projects designed to investigate whether gender differences in physiological responses to cold stress are of a sufficient magnitude to have implications for predictive models of human body temperature regulation.

In this study, one of the main objectives was to document the relative intensity of muscular contraction during shivering in a variety of skeletal muscle groups chosen to represent both central and distal body regions. In females, several muscle groups, (specifically, rectus femoris, rectus abdominis, pectoralis major and biceps brachii) were recruited and contracted continuously at low levels throughout cold air exposure. Neither the gastrocnemius nor the brachioradialis showed a significant increase in EMG activity during the cold air exposure compared to the pre-exposure rest. The peripheral muscles elicited low levels of EMG that were between 1 and 6% of their maximal capacity. The larger, central muscles elicited greater EMG levels that were between 10 and 14% of their maximal capacity. None of these muscles showed any further increase in their EMG activity over the course of the exposure. In male subjects, EMG activity during cold exposure was significantly elevated from pre-exposure in all muscle groups. Similarly to the females, males showed a significantly greater EMG activity in the central compared to the peripheral muscles however, in males, the larger muscle groups (gastrocnemius, rectus abdominus and pectoralis major) continued to increase their shivering intensity during the two hour exposure. In females, the muscle groups PE and AB accounted for 84.5% of the whole body measured EMG while FE contributed only about 5% and BB contributed less than 3.5%. In males, PE and AB also accounted for most of the measured EMG (70.5%) however, the FE contributed over 20%. These gender differences suggest

that females may be more effective than males in restricting the blood flow and the movement of the limbs and therefore reducing convective and radiative heat loss.

Heat transfer coefficient data appear to dispute this conclusion because h increased not only in PE but also in GA in both genders. In males however, the increases in h were consistent with the increases in EMG in these muscle groups. In females, while the increase in h in PE was associated with an increase in EMG, h in GA increased throughout exposure without any increase in EMG above pre-exposure values. This increase in h is best explained by lower leg movement due to muscle contraction of AB and FE. The shivering intensity of these two muscle groups did increase over the course of the cold exposure, albeit insignificantly, but the summation effect of these muscles on leg movement could account for the increase in the convective component of h.

Another objective was to relate the observed increase in muscle contraction to an increase in $\dot{M}$ . As in males, there was a strong, positive relationship (r=5.46) between metabolic heat production ( $\dot{M}$ per Kg LBM ) and whole body shivering intensity (SUM) in females. This correlation coefficient can be considered strong in light of the many measurement limitations to that correlation. Some limitations to a stronger relationship may include electrode placement, subcutaneous fat, recruitment of other muscles, and non-shivering thermogenesis.

Detailed analysis, interpretation of the results, and the implications for gender effect on predictive modeling will be reserved for the Final Report.

## 6 SUMMARY AND CONCLUSIONS

A. Data collection for the third phase of this project was completed in accordance with the experimental protocol. Vasoconstriction and lack of blood flow to the extremities resulted in slight difficulty in obtaining venous blood samples during cold air exposure.

B. In the present study with females, EMG activity during cold exposure was significantly greater than during pre-exposure in AB, PE, BB and FE but did not change during the course of the cold exposure. Shivering intensity was greatest in the central versus the peripheral muscles. In fact, AB and PE accounted for 84.5% of whole body measured EMG. There was no significant effect of cold exposure on EMG measured at BR or GA. Similarly, in males, EMG activity was also greatest in the trunk (PE and AB contributing 70.5%). In contrast to females, shivering EMG in males, was significantly higher than pre-exposure in all muscle groups measured and continued to increase throughout the exposure. Both males and females showed a strong positive correlation between metabolic heat production per lean body mass and whole body shivering.

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# 8 Tables.

Table 1. Subject Characteristics.

| Name | Age (yrs) | Height (cm) | Weight (Kg) | FAT (%) | BSA ($m^2$) | LBM (Kg) | Maximal Aerobic Power (ml/kg/min) | Menstrual Cycle Phase | Oral contraceptive |
|---|---|---|---|---|---|---|---|---|---|
| HC | 22 | 158.6 | 62.4 | 24.8 | 1.73 | 46.92 | 45.03 | 5 | none |
| JK | 34 | 167.5 | 57.2 | 19.6 | 1.69 | 46.00 | 38.81 | 8 | none |
| KK | 35 | 158.4 | 55.8 | 21.9 | 1.64 | 43.55 | 43.04 | 7 | none |
| DL | 35 | 168.0 | 59.9 | 20.8 | 1.73 | 47.44 | 27.88 | 4 | none |
| LM | 38 | 165.0 | 68.1 | 22.2 | 1.83 | 68.10 | 45.52 | 9 | none |
| LIM | 21 | 163.2 | 61.0 | 28.4 | 1.73 | 43.68 | 30.66 | 5 | none |
| DM | 30 | 161.6 | 57.2 | 23.3 | 1.67 | 43.88 | 27.27 | 6 | none |
| MM | 21 | 157.0 | 67.8 | 28.5 | 1.80 | 48.48 | 32.01 | 8 | triphasal 21 |
| BM | 29 | 154.2 | 59.6 | 22.8 | 1.68 | 46.01 | 38.59 | 7 | none |
| SP | 30 | 166.4 | 64.1 | 22.8 | 1.78 | 49.51 | 44.29 | 9 | none |
| BS | 35 | 170.0 | 76.5 | 29.9 | 1.95 | 53.65 | 26.26 | 9 | none |
| HS | 24 | 160.7 | 62.0 | 14.1 | 1.87 | 53.23 | 37.26 | 7 | triphasal 21 |
| SS | 23 | 168.0 | 70.0 | 23.0 | 1.73 | 53.90 | 34.86 | 10 | none |
| SY | 22 | 165.0 | 85.5 | 29.6 | 2.04 | 60.16 | 35.46 | 7 | none |
| LZ | 21 | 157.9 | 51.0 | 15.8 | 1.57 | 42.94 | 46.27 | 9 | none |
| n=15 | | | | | | | | | |
| Mean | 28.0 | 162.8 | 63.9 | 23.2 | 1.76 | 49.83 | 36.88 | 7.3 | |
| SD | 6.3 | 4.8 | 8.7 | 4.7 | 0.12 | 7.01 | 6.99 | 1.8 | |

Table 2. EMG (% MVC) during 30 min intervals of cold exposure.

| | EMG (% MVC) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | GA | | | | FE | | | | AB | | | |
| Subjects | 30 min | 60 min | 90 min | 120 min | 30 min | 60 min | 90 min | 120 min | 30 min | 60 min | 90 min | 120 min |
| JK | 1.55 | 2.76 | 2.63 | 1.57 | 0.00 | 0.03 | 0.45 | 0.16 | 2.23 | 18.72 | 34.48 | 22.56 |
| KK | 0.08 | 0.14 | 0.18 | 0.08 | 0.00 | 0.22 | 0.94 | 1.09 | 0.27 | 0.61 | 1.03 | 1.17 |
| DL | 1.15 | 0.72 | 5.65 | 4.01 | 0.17 | 0.30 | 0.62 | 0.14 | 0.76 | 0.72 | 1.12 | 1.02 |
| DM | 0.09 | 0.04 | 3.70 | 12.70 | 2.35 | 1.83 | 2.42 | 3.59 | 19.94 | 25.54 | 35.06 | 47.71 |
| BN | 0.00 | 0.26 | 0.05 | 0.62 | 0.20 | 0.42 | 2.15 | 2.12 | 10.08 | 15.39 | 15.38 | 11.24 |
| SP | 0.02 | 0.00 | 0.83 | 0.87 | 0.55 | 0.02 | 0.16 | 0.25 | 0.57 | 0.15 | 0.11 | 0.10 |
| HS | 0.00 | 0.01 | 0.04 | 0.07 | 0.00 | 0.01 | 1.12 | 4.47 | 0.69 | 0.99 | 1.54 | 1.16 |
| SS | 0.01 | 0.03 | 0.00 | 0.00 | 0.43 | 0.45 | 0.31 | 1.39 | 4.12 | 5.40 | 7.64 | 6.65 |
| LZ | 0.00 | 0.01 | 0.01 | 0.01 | 0.61 | 3.40 | 10.49 | 11.64 | 0.65 | 2.12 | 0.91 | 2.33 |
| HC | 0.00 | 0.03 | 0.13 | 0.00 | 0.06 | 0.50 | 0.26 | 0.14 | 18.57 | 25.25 | 28.00 | 28.50 |
| LM | 0.01 | 0.00 | 0.03 | 0.00 | 0.04 | 0.00 | 0.03 | 0.03 | 24.61 | 17.92 | 29.24 | 33.27 |
| LIM | 0.00 | 0.00 | 0.19 | 0.04 | 1.08 | 2.36 | 7.03 | 7.20 | 23.23 | 32.61 | 31.54 | 30.64 |
| MM | 0.90 | 1.16 | 0.53 | 2.06 | 0.25 | 0.07 | 0.21 | 0.06 | 8.17 | 9.87 | 6.35 | 3.09 |
| BS | 0.00 | 0.46 | 1.87 | 1.37 | 0.00 | 0.01 | 0.01 | 0.01 | 7.56 | 8.39 | 8.12 | 8.04 |
| SY | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.04 | 0.01 | 0.00 | 0.18 | 0.28 | 0.05 |
| Mean | 0.25 | 0.37 | 1.06 | 1.56 | 0.38 | 0.64 | 1.75* | 2.15* | 8.10 | 10.92 | 13.39* | 13.17* |
| SD | 0.51 | 0.74 | 1.70 | 3.28 | 0.63 | 1.04 | 3.01 | 3.36 | 9.08 | 10.94 | 14.07 | 15.34 |

*** different from 30 min ($p<0.05$)**

Table 3. EMG (% MVC) during 30 min intervals of cold exposure.

| | EMG (%CONTRIBUTION) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PE | | | | BB | | | | BR | | | |
| Subjects | 30 min | 60 min | 90 min | 120 min | 30 min | 60 min | 90 min | 120 min | 30 min | 60 min | 90 min | 120 min |
| JK | N/A | N/A | N/A | N/A | 1.32 | 1.41 | 2.23 | 3.05 | 0.34 | 0.87 | 1.61 | 2.31 |
| KK | 4.64 | 13.96 | 18.24 | 20.04 | 0.00 | 0.05 | 0.53 | 0.58 | 0.01 | 0.14 | 0.21 | 0.52 |
| DL | 10.88 | 10.46 | 12.80 | 12.40 | 0.53 | 0.14 | 1.88 | 1.27 | 1.37 | 0.94 | 2.47 | 0.64 |
| DM | 2.63 | 3.79 | 4.12 | 3.19 | 0.67 | 1.15 | 0.85 | 1.36 | 1.45 | 0.91 | 0.50 | 0.58 |
| BN | 3.38 | 6.07 | 6.59 | 5.29 | 0.54 | 1.96 | 1.84 | 1.10 | 1.75 | 3.44 | 2.76 | 2.42 |
| SP | 13.94 | 10.02 | 12.65 | 14.08 | 1.60 | 0.23 | 0.64 | 0.94 | 0.30 | 0.16 | 0.22 | 0.28 |
| HS | 1.38 | 2.38 | 3.96 | 4.28 | 0.00 | 0.02 | 0.24 | 0.45 | 0.19 | 0.10 | 0.02 | 0.24 |
| SS | 17.68 | 17.73 | 19.14 | 19.85 | 0.56 | 0.10 | 0.01 | 0.09 | 0.31 | 0.48 | 0.07 | 0.01 |
| LZ | 1.15 | 4.84 | 8.05 | 7.92 | 0.00 | 0.04 | 0.08 | 0.20 | 0.02 | 0.51 | 0.66 | 0.86 |
| HC | 3.93 | 12.03 | 11.98 | 11.32 | 0.04 | 1.09 | 1.18 | 0.99 | 0.11 | 0.99 | 1.58 | 1.51 |
| LM | 13.70 | 13.26 | 12.86 | 13.67 | 2.76 | 1.80 | 2.44 | 3.33 | 0.28 | 0.00 | 0.42 | 0.36 |
| LIM | 14.52 | 13.35 | 19.54 | 17.11 | 0.42 | 1.51 | 3.25 | 2.85 | 0.07 | 1.36 | 2.73 | 1.56 |
| MM | 5.59 | 6.46 | 11.18 | 7.68 | 0.43 | 0.78 | 1.39 | 1.03 | 0.35 | 0.71 | 1.40 | 0.57 |
| BS | 3.27 | 1.82 | 1.69 | 2.35 | 0.48 | 0.01 | 0.02 | 0.16 | 1.57 | 0.28 | 0.39 | 0.18 |
| SY | 0.20 | 0.14 | 2.36 | 2.28 | 0.01 | 0.09 | 0.03 | 0.02 | 0.00 | 0.10 | 0.14 | 0.05 |
| Mean | 6.92 | 8.31 | 10.37* | 10.11* | 0.62 | 0.69 | 1.11* | 1.16* | 0.54 | 0.73 | 1.01 | 0.81 |
| SD | 5.91 | 5.38 | 6.09 | 6.29 | 0.76 | 0.73 | 1.03 | 1.08 | 0.64 | 0.85 | 1.00 | 0.78 |

* different from 30 min ($p<0.05$)

Table 4. EMG (% contribution) during 30 min intervals of cold exposure.

| | EMG (% CONTRIBUTION) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | GA | | | | FE | | | | AB | | | |
| Subjects | 30 min | 60 min | 90 min | 120 min | 30 min | 60 min | 90 min | 120 min | 30 min | 60 min | 90 min | 120 min |
| JK | 31.29 | 11.78 | 6.21 | 5.16 | 0.07 | 0.10 | 1.07 | 0.48 | 19.91 | 39.80 | 41.79 | 38.20 |
| KK | 2.33 | 0.79 | 0.84 | 0.37 | 0.00 | 1.48 | 4.43 | 4.66 | 4.30 | 3.96 | 4.81 | 4.94 |
| DL | 8.82 | 4.16 | 21.29 | 19.74 | 1.37 | 2.02 | 2.55 | 0.78 | 5.43 | 5.46 | 4.73 | 5.33 |
| DM | 0.14 | 0.09 | 6.19 | 18.28 | 7.35 | 5.41 | 5.45 | 5.18 | 79.45 | 76.44 | 74.83 | 69.16 |
| BN | 0.00 | 1.00 | 0.18 | 2.59 | 0.98 | 1.48 | 7.33 | 8.33 | 64.34 | 55.97 | 53.56 | 50.08 |
| SP | 0.09 | 0.01 | 5.60 | 5.50 | 2.71 | 0.15 | 1.08 | 1.37 | 3.22 | 1.43 | 0.74 | 0.64 |
| HS | 0.00 | 0.19 | 0.38 | 0.64 | 0.00 | 0.17 | 11.38 | 39.70 | 29.60 | 24.47 | 19.35 | 10.53 |
| SS | 0.02 | 0.16 | 0.00 | 0.00 | 2.10 | 2.00 | 1.15 | 4.89 | 17.99 | 22.02 | 28.03 | 23.30 |
| LZ | 0.00 | 0.06 | 0.06 | 0.05 | 26.32 | 30.20 | 50.11 | 50.65 | 20.61 | 19.50 | 4.57 | 8.64 |
| HC | 0.00 | 0.08 | 0.30 | 0.01 | 0.24 | 1.26 | 0.60 | 0.33 | 79.36 | 63.36 | 64.99 | 67.41 |
| LM | 0.04 | 0.00 | 0.06 | 0.00 | 0.09 | 0.00 | 0.06 | 0.05 | 60.12 | 52.89 | 65.02 | 65.44 |
| LIM | 0.00 | 0.00 | 0.28 | 0.07 | 2.47 | 4.05 | 10.67 | 12.01 | 59.65 | 65.23 | 49.23 | 51.70 |
| MM | 6.59 | 5.77 | 2.56 | 13.57 | 1.09 | 0.39 | 0.95 | 0.42 | 55.08 | 51.84 | 29.42 | 22.26 |
| BS | 0.00 | 3.74 | 12.78 | 8.86 | 0.00 | 0.08 | 0.04 | 0.09 | 62.24 | 78.59 | 71.52 | 68.98 |
| SY | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 1.31 | 0.55 | 0.03 | 36.79 | 7.36 | 5.67 |
| Mean | 3.29 | 1.86 | 3.78 | 4.99 | 2.99 | 3.25 | 6.55 | 8.63 | 37.42 | 39.85 | 34.66 | 32.82 |
| SD | 8.21 | 3.31 | 6.08 | 6.95 | 6.73 | 7.62 | 12.62 | 15.38 | 29.16 | 26.10 | 27.16 | 26.94 |

Table 5. EMG (% contribution) during 30 min intervals of cold exposure.

| | EMG (% CONTRIBUTION) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PE | | | | BB | | | | BR | | | |
| Subjects | 30 min | 60 min | 90 min | 120 min | 30 min | 60 min | 90 min | 120 min | 30 min | 60 min | 90 min | 120 min |
| JK | 19.91 | 39.80 | 41.79 | 38.20 | 21.19 | 4.56 | 5.21 | 10.05 | 7.63 | 3.95 | 3.92 | 7.92 |
| KK | 93.21 | 92.45 | 86.45 | 85.44 | 0.01 | 0.30 | 2.47 | 2.44 | 0.15 | 1.01 | 1.00 | 2.15 |
| DL | 72.81 | 80.62 | 53.88 | 65.27 | 3.43 | 0.95 | 6.91 | 5.85 | 8.15 | 6.79 | 10.63 | 3.03 |
| DM | 10.58 | 11.83 | 9.96 | 4.60 | 2.23 | 3.55 | 2.39 | 1.94 | 0.25 | 2.68 | 1.17 | 0.84 |
| BN | 20.58 | 21.98 | 23.14 | 24.35 | 3.48 | 7.08 | 6.12 | 3.94 | 10.63 | 12.49 | 9.67 | 10.71 |
| SP | 84.22 | 94.69 | 86.72 | 85.33 | 8.25 | 2.14 | 4.36 | 5.43 | 1.51 | 1.59 | 1.50 | 1.73 |
| HS | 61.42 | 72.40 | 65.66 | 42.35 | 0.00 | 0.37 | 3.00 | 4.09 | 8.98 | 2.40 | 0.23 | 2.69 |
| SS | 76.17 | 73.18 | 70.49 | 71.48 | 2.69 | 0.44 | 0.05 | 0.28 | 1.03 | 2.21 | 0.28 | 0.05 |
| LZ | 52.13 | 45.01 | 41.45 | 36.13 | 0.00 | 0.42 | 0.39 | 0.81 | 0.95 | 4.81 | 3.41 | 3.72 |
| HC | 19.62 | 30.13 | 27.77 | 26.52 | 0.20 | 2.71 | 2.71 | 2.28 | 0.59 | 2.47 | 3.63 | 3.47 |
| LM | 32.55 | 41.72 | 28.51 | 27.13 | 6.46 | 5.39 | 5.31 | 6.63 | 0.75 | 0.00 | 1.04 | 0.75 |
| LIM | 36.52 | 25.77 | 30.56 | 28.81 | 1.21 | 2.60 | 5.02 | 4.80 | 0.16 | 2.35 | 4.24 | 2.62 |
| MM | 32.99 | 34.29 | 53.60 | 52.91 | 2.09 | 4.03 | 6.62 | 6.98 | 2.17 | 3.69 | 6.85 | 3.86 |
| BS | 24.31 | 15.22 | 12.11 | 19.93 | 2.60 | 0.09 | 0.12 | 1.01 | 10.84 | 2.29 | 3.43 | 1.14 |
| SY | 98.15 | 61.15 | 89.08 | 91.39 | 1.83 | 5.84 | 0.71 | 0.65 | 0.00 | 1.22 | 1.55 | 1.74 |
| Mean | 49.01 | 49.35 | 48.08 | 46.65 | 3.71 | 2.70 | 3.43 | 3.81 | 3.58 | 3.33 | 3.50 | 3.09 |
| SD | 29.72 | 27.74 | 26.83 | 27.11 | 5.37 | 2.30 | 2.41 | 2.83 | 4.25 | 3.02 | 3.25 | 2.82 |

Table 6. EMG SUM during exposure.

| | SUM | | | |
|---|---|---|---|---|
| Subject | 30 min | 60 min | 90 min | 120 min |
| JK | 1.58 | 10.28 | 18.82 | 12.40 |
| KK | 1.70 | 4.94 | 6.73 | 7.41 |
| DL | 4.17 | 3.88 | 5.42 | 4.89 |
| DM | 4.75 | 6.78 | 9.15 | 12.37 |
| BN | 3.26 | 5.37 | 6.00 | 4.75 |
| SP | 4.89 | 3.46 | 4.48 | 5.02 |
| HS | 0.68 | 1.01 | 1.98 | 3.01 |
| SS | 7.47 | 7.21 | 8.05 | 8.42 |
| LZ | 0.75 | 3.06 | 5.98 | 6.55 |
| HC | 5.04 | 9.13 | 9.60 | 9.42 |
| LM | 9.19 | 8.02 | 10.10 | 11.19 |
| LIM | 9.81 | 11.56 | 14.98 | 13.96 |
| MM | 3.60 | 4.26 | 5.24 | 3.47 |
| BS | 2.56 | 2.26 | 2.29 | 2.46 |
| SY | 0.07 | 0.09 | 0.87 | 0.79 |
| mean | 3.97 | 5.42 | 7.31* | 7.07* |
| SD | 3.00 | 3.36 | 4.80 | 4.08 |

* different from 30 min ($p<0.005$)

**Table 7. Metabolic rate before and during 30 min intervals of cold air exposure.**

| Subject | Metabolic Rate ($W/m^2$) | | | | |
|---|---|---|---|---|---|
| | Pre | 30 min | 60 min | 90 min | 120 min |
| JK | 45.5 | 45.6 | 75.2 | 96.3 | 83.7 |
| KK | 41.7 | 47.9 | 62.2 | 79.5 | 77.9 |
| DL | 41.4 | 58.9 | 63.5 | 83.8 | 78.7 |
| DM | 28.7 | 64.5 | 67.3 | 115.2 | 104.6 |
| BN | 28.7 | 46.3 | 61.3 | 67.6 | 66.7 |
| SP | 37.1 | 84.6 | 63.1 | 71.1 | 75.3 |
| HS | 35.6 | 55.8 | 54.7 | 64.8 | 75.9 |
| SS | 39.9 | 61.0 | 61.3 | 65.1 | 71.4 |
| LZ | 39.2 | 53.3 | 63.5 | 75.5 | 79.9 |
| HC | 35.7 | 60.0 | 74.0 | 76.5 | 77.6 |
| LM | 28.5 | 63.2 | 47.7 | 65.8 | 73.4 |
| LIM | 40.7 | 80.0 | 79.8 | 91.1 | 87.5 |
| MM | 36.8 | 45.2 | 48.5 | 61.6 | 51.7 |
| BS | 38.3 | 54.5 | 50.6 | 52.7 | 48.8 |
| SY | 37.8 | 44.9 | 41.8 | 41.6 | 44.6 |
| **Mean** | 37.0 | 57.7* | 60.97* | 73.88*+ | 73.17*+ |
| **SD** | 5.1 | 12.1 | 10.8 | 18.0 | 15.5 |

*different from Pre ($p<0.001$)

+ different from 60 min ($p<0.005$)

Table 8. Core temperature before and during 30 min intervals during exposure.

| Subject | Core Temperature (°C) | | | | |
|---|---|---|---|---|---|
| | Pre | 30 min | 60 min | 90 min | 120 min |
| JK | 36.93 | 37.06 | 37.01 | 36.85 | 36.89 |
| KK | 37.01 | 37.07 | 37.07 | 36.99 | 36.98 |
| DL | 36.79 | N/A | N/A | N/A | N/A |
| DM | 36.99 | 37.20 | 37.49 | 37.46 | 37.37 |
| BN | 36.73 | 36.92 | 37.09 | 37.08 | 37.13 |
| SP | 36.70 | 36.93 | 37.40 | 37.47 | 37.43 |
| HS | 36.63 | N/A | N/A | N/A | N/A |
| SS | 36.73 | 36.57 | 36.84 | 36.75 | 36.68 |
| LZ | 36.73 | 36.92 | 37.09 | 37.08 | 37.13 |
| HC | 37.04 | 37.19 | 37.29 | 37.28 | 37.21 |
| LM | 37.13 | 37.31 | 37.59 | 37.43 | 37.23 |
| LIM | 37.00 | 37.09 | 37.31 | 37.29 | 37.30 |
| MM | 37.04 | 37.16 | 37.29 | 37.20 | 37.05 |
| BS | 36.86 | 37.03 | 37.33 | 37.30 | 37.21 |
| SY | 37.01 | 37.10 | 37.20 | 37.15 | 37.01 |
| Mean | 36.89 | 37.04 | 37.23* | 37.18* | 37.12* |
| SD | 0.16 | 0.18 | 0.21 | 0.23 | 0.20 |

* different from Pre ($p<0.001$)

Table 9. Heat transfer coefficient during the initial 5 min and 30 min intervals of a 2-hour cold air exposure.

| Subject | Heat Transfer Coefficient (GA) | | | | | Heat Transfer Coefficient (PE) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 5 min | 30 min | 60 min | 90 min | 120 min | 5 min | 30 min | 60 min | 90 min | 120 min |
| JK | 3.599 | 4.26 | 5.40 | 5.65 | 6.28 | 8.944 | 8.61 | 8.23 | 7.92 | 7.74 |
| KK | 5.861 | 5.55 | 4.95 | 5.35 | 5.45 | 8.690 | 8.79 | 9.24 | 9.36 | 9.33 |
| DL | 4.114 | 4.60 | 5.64 | 5.17 | 4.81 | 9.935 | 4.54 | 8.34 | 9.45 | 10.85 |
| DM | 3.210 | 4.04 | 4.74 | 5.37 | 5.39 | 9.976 | 8.30 | 8.76 | 8.67 | 8.86 |
| BN | 3.423 | 3.69 | 4.29 | 4.65 | 4.59 | 7.650 | 7.86 | 7.33 | 8.35 | 8.19 |
| SP | 3.476 | 3.99 | 4.50 | 4.98 | 6.18 | 7.651 | 7.87 | 7.90 | 7.27 | 8.24 |
| HS | 3.861 | 4.08 | 4.58 | 4.61 | 5.31 | 3.871 | 4.00 | 6.43 | 6.63 | 7.32 |
| SS | 4.233 | 4.78 | 5.34 | 5.70 | 6.42 | 7.948 | 5.06 | 9.67 | 7.53 | 7.92 |
| LZ | 3.423 | 3.69 | 4.29 | 4.65 | 4.59 | 7.650 | 7.86 | 7.33 | 8.35 | 8.19 |
| HC | 3.063 | 3.41 | 4.91 | 5.34 | 5.78 | 8.233 | 7.70 | 7.53 | 6.29 | 7.24 |
| LM | 3.136 | 3.38 | 5.62 | 6.08 | 5.61 | 7.476 | 7.24 | 7.48 | 8.53 | 8.02 |
| LIM | 2.978 | 3.78 | 5.52 | 5.75 | 5.51 | 7.950 | 7.44 | 7.32 | 6.91 | 6.83 |
| MM | 4.928 | 5.58 | 5.92 | 5.67 | 6.12 | 11.772 | 10.88 | 11.83 | 11.54 | 10.29 |
| BS | 4.670 | 4.76 | 6.26 | 6.58 | 4.94 | 4.956 | 4.35 | 4.07 | 3.75 | 3.90 |
| SY | 3.540 | 4.45 | 4.24 | 4.68 | 5.36 | 10.595 | 8.62 | 9.07 | 10.56 | 10.06 |
| Mean | 3.83 | 4.27* | 5.08*+ | 5.35* | 5.49* | 8.22 | 7.27 | 8.04 | 8.07 | 8.20 |
| SD | 0.80 | 0.69 | 0.64 | 0.58 | 0.59 | 2.00 | 1.94 | 1.70 | 1.87 | 1.67 |

* different from 5 min ($p<0.05$)

+ different from 30 min ($p<0.001$).

**Table 10. Blood metabolite and hormone responses to 2-hour cold air exposure.**

| | Hemaglobin(g/dL) | | Hematocrit | | Lactate (mmol/l) | | Glucose (mg/dl) | | NEFA (mEq/l) | | Glycerol (mmol/l) | | PV delta |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Subjects | Pre | Post | Pre | Post | Pre | Post | Pre | Post | Pre | Post | Pre | Post | Post |
| JK | 11.6 | 13 | 34 | 39 | 0.4 | 1 | 56.56 | 62.26 | 0.5 | 1.16 | 0.041 | 0.23 | -17.5 |
| KK | 10.4 | | 33 | | 0.8 | | 62.61 | | 0.34 | | 0.026 | | |
| DL | 12 | 13.4 | 35 | 38 | 0.5 | 1.1 | 66.07 | 67.8 | 0.74 | 0.52 | 0.094 | 0.055 | -14.6 |
| DM | 13.7 | 15.3 | 40 | 46 | 0.7 | 2.1 | 61.4 | 63.47 | 0.53 | 1.29 | 0.046 | 0.212 | -19.4 |
| BN | 13 | 14.2 | 37 | 40 | 0.6 | 1.1 | 63.65 | 62.44 | 0.45 | 1.51 | 0.045 | 0.322 | -12.8 |
| SP | 13.9 | 15 | 39 | 45 | 0.5 | 1.4 | 65.03 | 72.64 | 0.42 | 0.71 | 0.067 | 0.099 | -16.5 |
| HS | 12.3 | 13.8 | 37 | 42 | 0.7 | 0.7 | 62.78 | 56.04 | 0.61 | 1.04 | 0.069 | 0.181 | -17.9 |
| SS | 12.8 | 14.2 | 37 | 39 | 0.7 | 1.5 | 60.19 | 59.5 | 0.69 | 1.46 | 0.088 | 0.299 | -12.7 |
| LZ | 13 | 14 | 38 | 41 | 0.7 | 2 | 61.57 | 51.71 | 0.45 | 1.24 | 0.031 | 0.229 | -11.6 |
| HC | 13.4 | 15.1 | 38 | 44 | 0.8 | 1.5 | 72.12 | 66.24 | 0.47 | 1.11 | 0.06 | 0.171 | -19.9 |
| LM | 12.5 | 13.2 | 35 | 40 | 0.3 | 0.7 | 56.21 | 54.83 | 0.53 | 0.98 | 0.026 | 0.139 | -12.6 |
| LIM | 13.2 | 14.8 | 37 | 42 | 0.7 | 2 | 58.81 | 62.09 | 0.75 | 1.66 | 0.087 | 0.283 | -17.9 |
| MM | 11.4 | 12.8 | 36 | 38 | 0.9 | 1.2 | 58.81 | 51.37 | 0.85 | 1.1 | 0.119 | 0.259 | -13.7 |
| BS | 12.4 | 13.2 | 36 | 40 | 0.4 | 0.5 | 57.59 | 57.08 | 0.53 | 0.9 | 0.031 | 0.124 | -11.9 |
| SY | 12.8 | 14.2 | 38 | 43 | 0.7 | 0.6 | 69.87 | 65.72 | 0.51 | 0.81 | 0.068 | 0.109 | -17.1 |
| Mean | 12.56 | 14.01* | 36.67 | 41.21* | 0.63 | 1.24* | 62.22 | 60.94 | 0.56 | 1.11* | 0.06 | 0.19* | -15.44 |
| SD | 0.93 | 0.82 | 1.88 | 2.55 | 0.17 | 0.53 | 4.63 | 6.21 | 0.14 | 0.32 | 0.03 | 0.08 | 2.89 |

* different from Pre ($p<0.001$)

## 9 Figures.

Figure 1. Mean emg for each muscle group averaged over 30 min intervals during cold exposure.

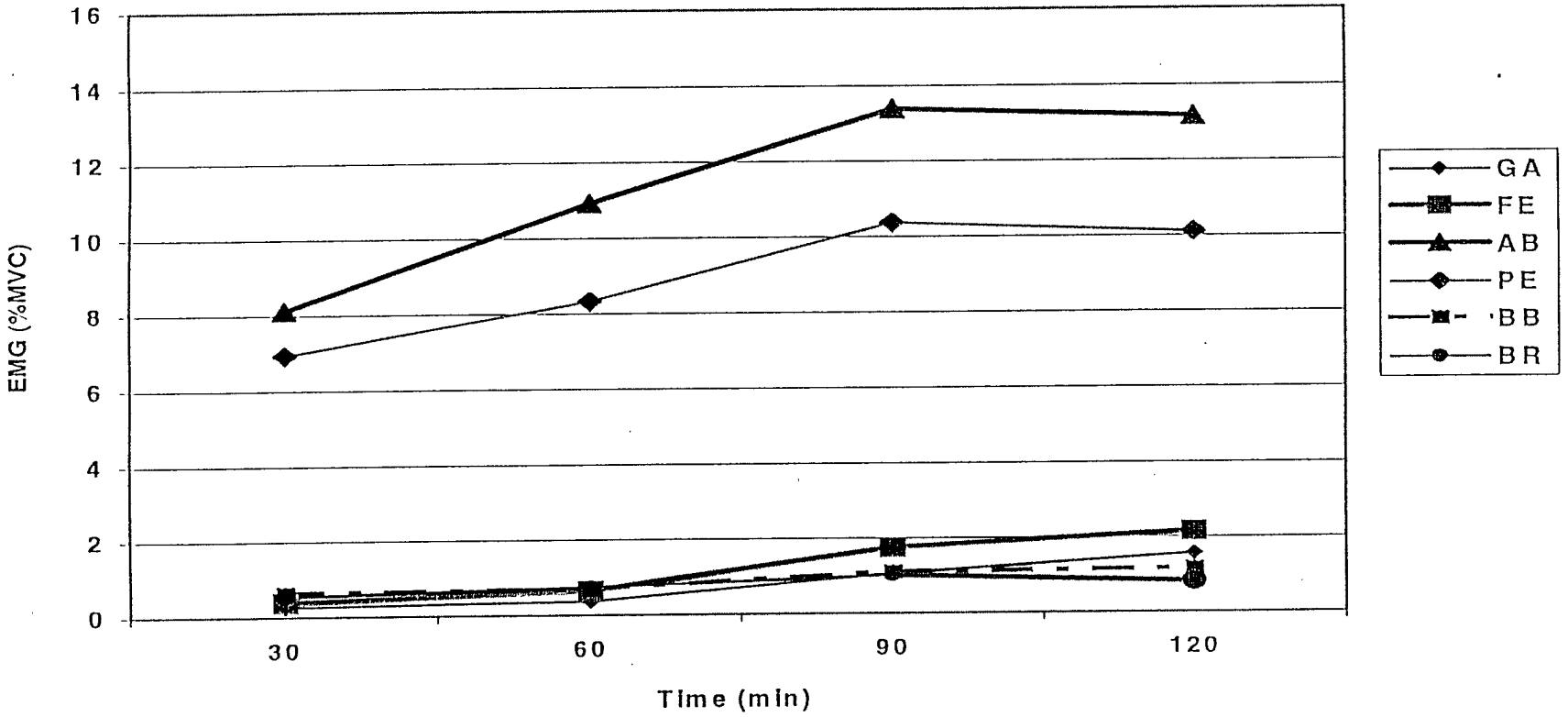


Figure 2. Mean EMG (% Contribution) during cold air exposure.

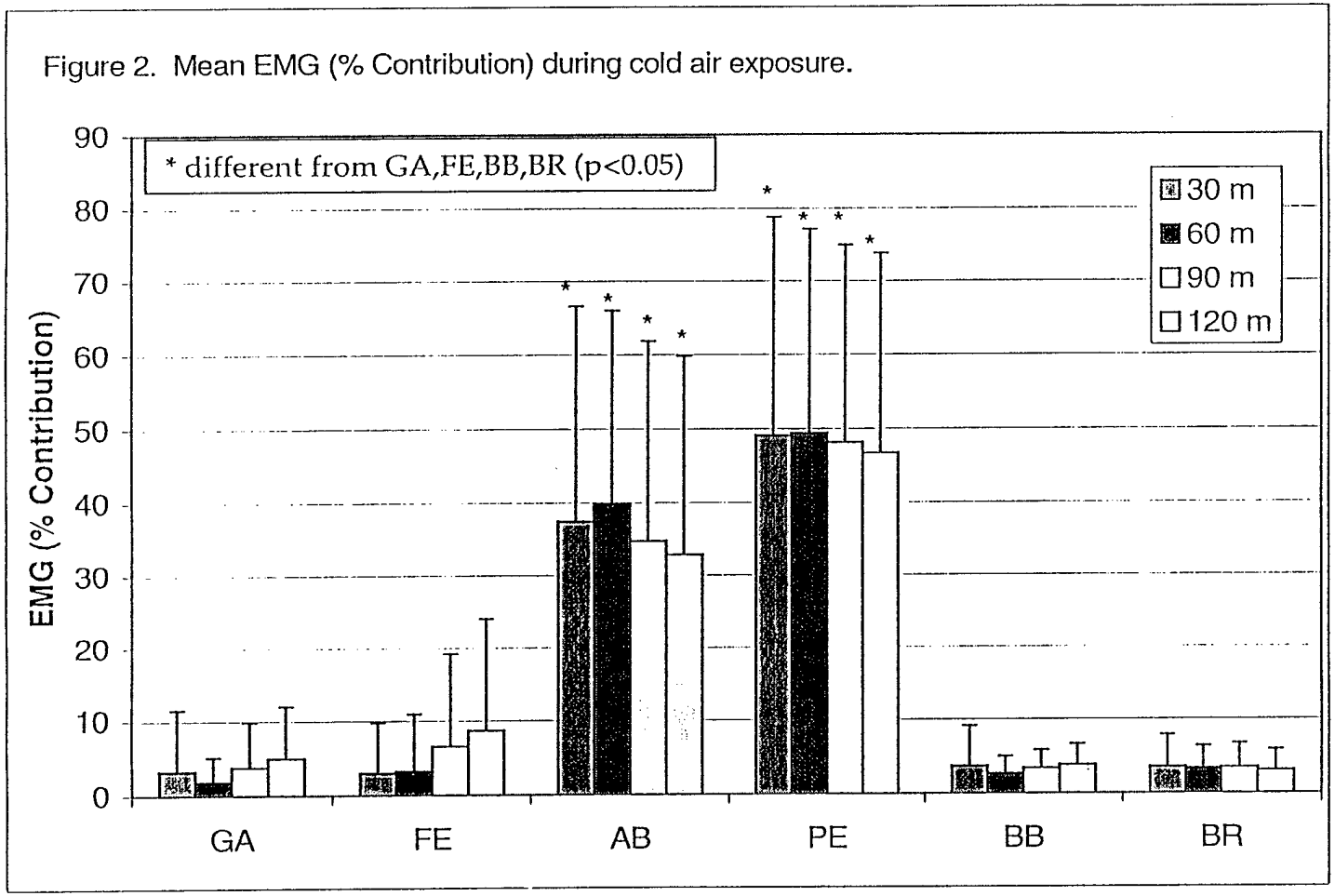


Figure 3. Mean M before and during cold air exposure.

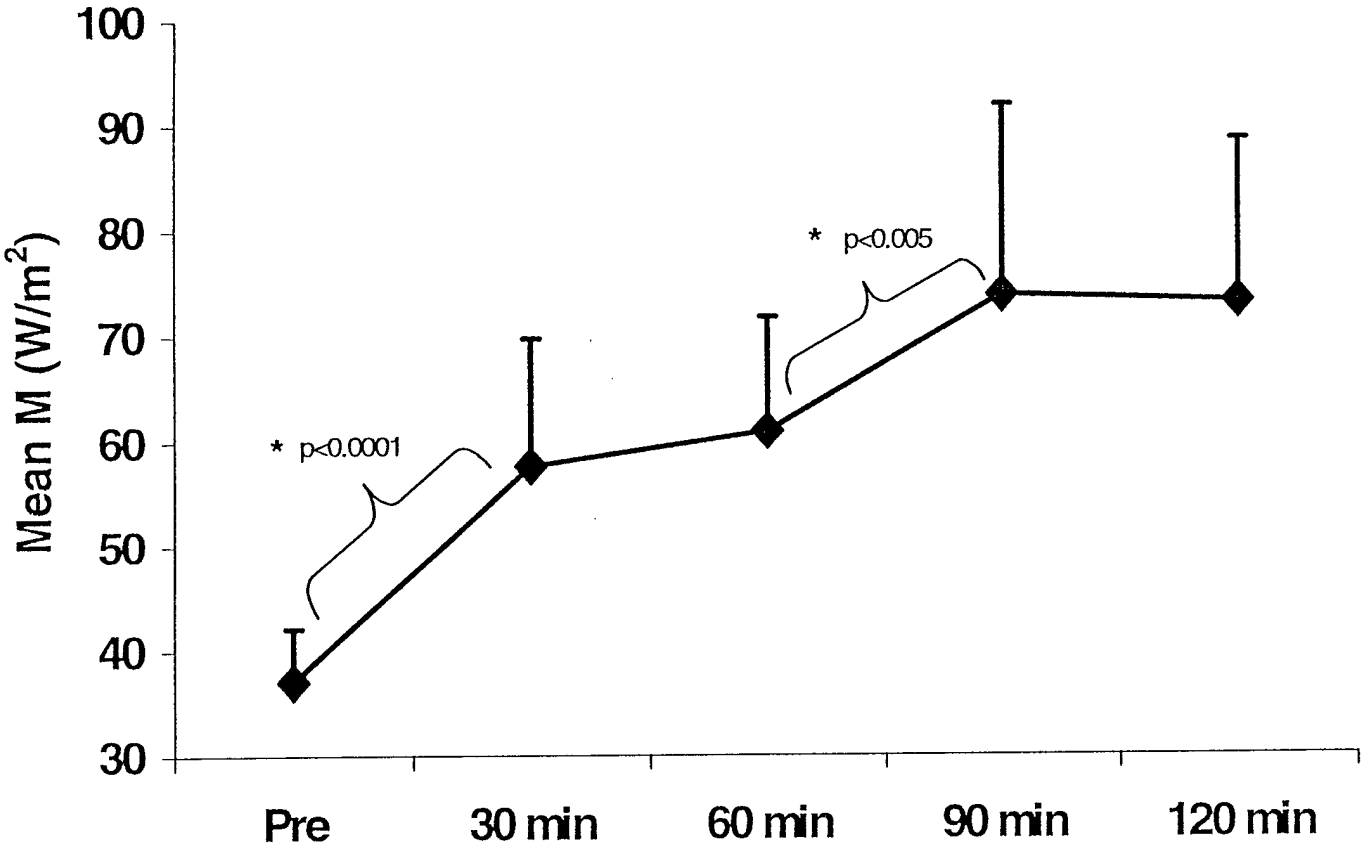


Figure 4. Relationship between metabolic heat production and whole body shivering intensity.

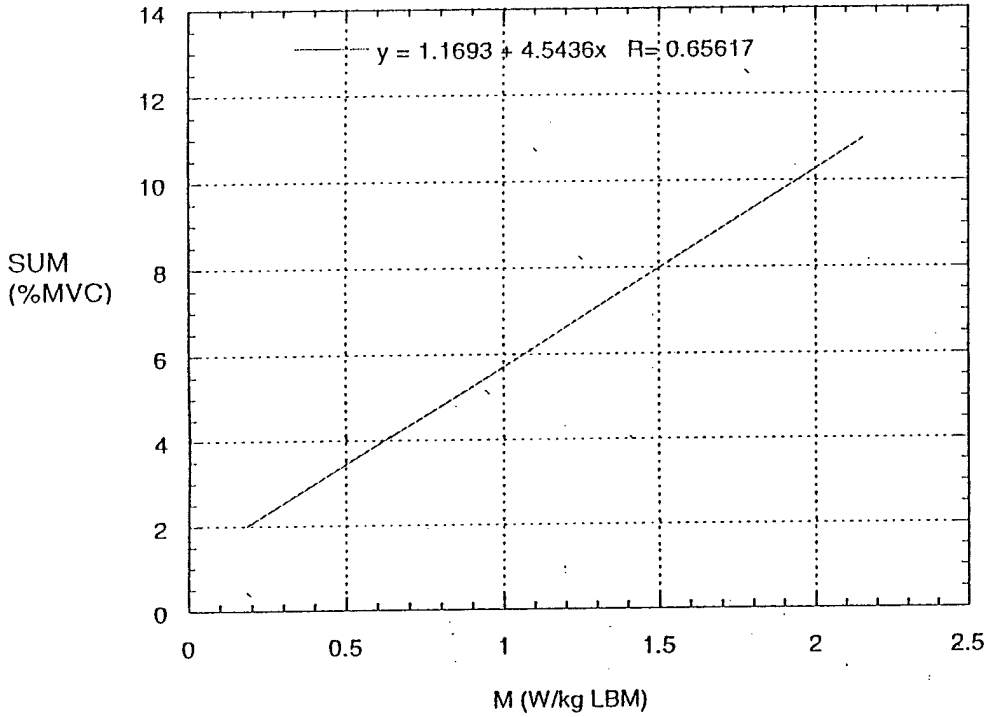
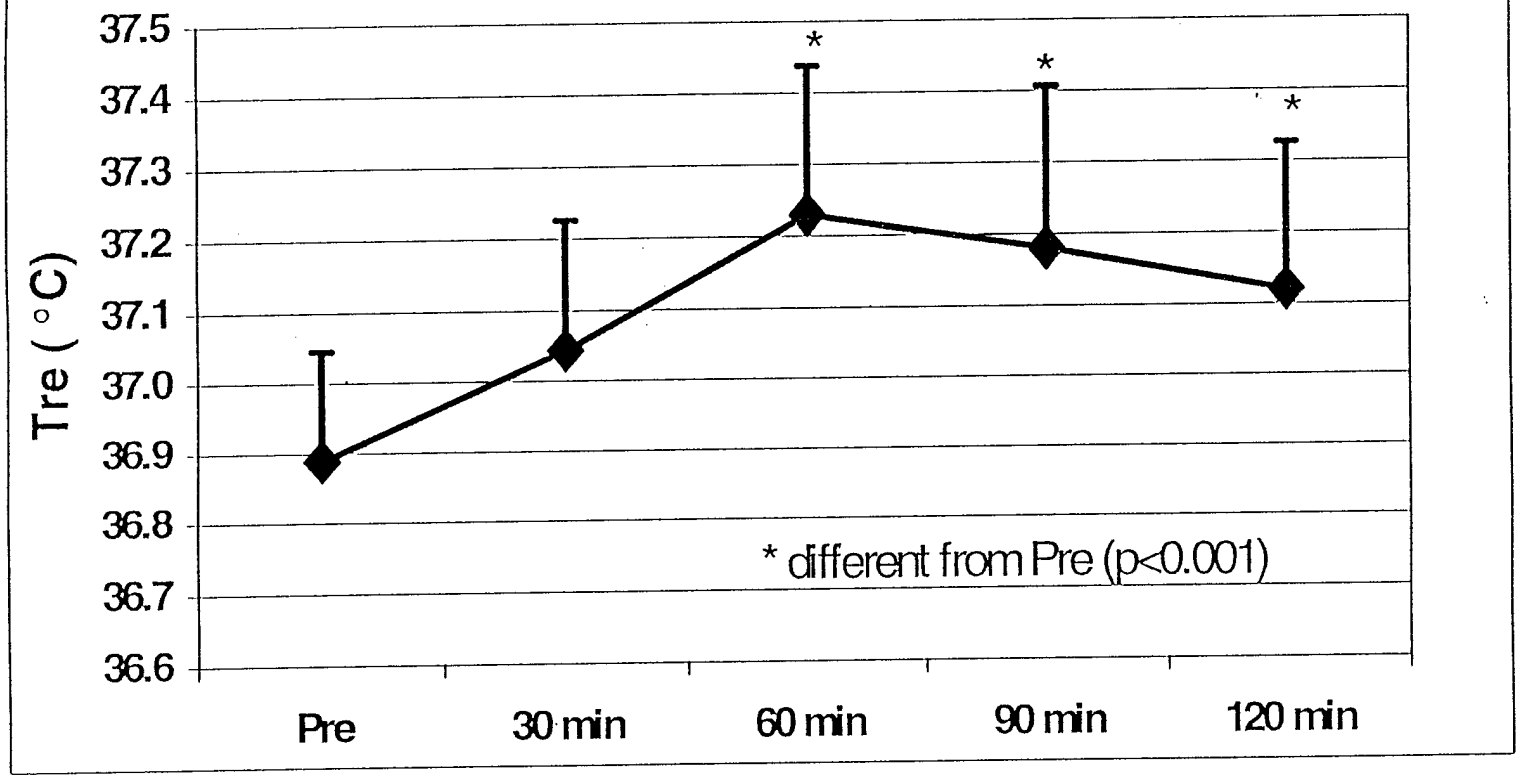


Figure 5. Mean core temperature response before and during cold air exposure.



## ANNEX D

**Salaried Personnel.**

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**Presentations, Abstracts, Publications to date:**

Jacobs I, Tikuisis P, Moroz D, Vallerand A. Changes in metabolic rate and rectal temperature in females during shivering in 18°C water. Canadian Society of Exercise Physiology 1997, Toronto, ON.

Jacobs I, Moroz D, Tikuisis P, Vallerand A. Changes in muscle glycogen and blood metabolites after cold water immersion in females. Abstract. 8th ICEE 1998, San Diego, USA.

Moroz D, Jacobs I, Vallerand A, Tikuisis P. Shivering thermogensesis in females after changes in dietary carbohydrate intake. Abstract. 8th ICEE 1998, San Diego, USA.

Tikuisis P, Jacobs I, Moroz D, Vallerand A, Martineau L. Female versus male thermoregulatory responses to immersion in 18°C water. Abstract. 9th ICEE Ruhr 2000, Dortmund, Germany.

Tikuisis P, Jacobs I, Moroz D, Vallerand A, Bell D. Female vs. male shivering EMG responses to 10°C air. Abstract. 9th ICEE Ruhr 2000, Dortmund, Germany.

Tikuisis P, Jacobs I, Moroz D, Vallerand A, Martineau L. Comparison of thermoregulatory responses between males and females immersed in cold water. Submitted.